

REMARKS

This Amendment and Reply accompanies applicant's Request for Continuing Examination (RCE) and addresses issues raised by the Examiner in the outstanding final rejection mailed July 26, 2004. A Petition for a one month extension of time for response to the outstanding action and the requisite fee accompany this RCE and Amendment and Reply, thereby extending the period for response until November 26, 2004. A Supplemental Information Disclosure Statement citing several references also accompanies this RCE and Amendment and Reply.

Claim 21 has been amended to recite a method for treating migraine headache and symptoms of migraine headaches in a human subject in need thereof comprising administering an effective amount of a treatment composition comprising a $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter antagonist capable of inhibiting $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransport in glial cells to the CNS of the subject. This amendment deletes reference to cortical spreading depression and incorporates subject matter previously recited in dependent claims 24 and 30. Claims 23 and 24 have been cancelled. Claim 27 has been amended to depend from independent claim 21 and recite that the loop diuretic is selected from the group consisting of furosemide, furosemide-related compositions, bumetanide, and ethacrynic acid. Exemplary loop diuretics are described in applicant's specification, for example, at the paragraph bridging pages 10 and 11. Claim 28 has been amended to depend from independent claim 21 and claims 30 and 31 have been cancelled.

Claim 35 has been amended to recite a method for reversing prolonged migraine aura in a human comprising administering a $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter antagonist capable of inhibiting $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransport in glial cells to the CNS in an amount effective in ameliorating or aborting the prolonged migraine aura. This aspect of applicant's claimed invention is described in the specification as filed, for example, at page 21. Claims 36 and 37 are amended for purposes of clarification.

Claim 38 has been amended to delete reference to cortical spreading depression and to recite a method for treating a human patient comprising administering an effective therapeutic amount of a treatment composition comprising a loop diuretic using a delivery regimen that provides an effective therapeutic amount of the loop diuretic to the CNS. The importance of delivering the treatment composition using a delivery regimen that provides an effective

therapeutic amount in the CNS is described in applicant's specification, for example, at page 17, lines 12-31. Claims 41 and 43 have been amended to conform to amendments made to claim 21.

Claims 46-55 have been added. Claim 46 specified that the cation chloride cotransporter antagonist comprises thiazide or a thiazide-like composition. This aspect of applicant's claimed invention is described in the specification, for example, at the paragraph spanning pages 10 and 11. Claim 47 specifies that the treatment composition is administered transdermally. This aspect of applicant's claimed invention is described in the specification, for example, at the paragraph spanning pages 15 and 16. Claim 48 specifies that the treatment composition is administered in a sustained release formulation. This aspect of applicant's claimed invention is described in the specification, for example, at the paragraph spanning pages 16 and 17. Claim 49 specifies that the treatment composition is administered in a dosage incorporated in a non-reactive carrier. This aspect of applicant's claimed invention is described in the specification, for example, at page 16 lines 21-28. Claim 50 specifies that the treatment composition is delivered in a liposome formulation. This aspect of applicant's claimed invention is described in the specification, for example, at page 17, lines 21-25.

Claim 51 specifies that the treatment composition is administered by implantation of a formulation or therapeutic device at one or more target sites for delivery of the treatment composition to the CNS and claim 51 specifies that the formulation or therapeutic device is actuatable externally upon the onset of symptoms. These aspects of applicant's claimed invention are described in the specification, for example, at page 16, lines 21-28 and at page 9, lines 9-12. Claim 53 specifies that the treatment composition is administered with a hyperosmotic agent. This aspect of applicant's claimed invention is described in the specification, for example, at page 15 lines 32-34. Claims 54 and 55 specify that the treatment composition consists essentially of a $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter antagonist or loop diuretic, respectively. These aspects of applicant's claimed invention are described throughout the application as filed.

It is urged that there is a clear basis in the specification, as filed, for the claim amendments and the newly added claims and that no new matter has been added.

Claim Rejections – 35 USC § 112

Claims 21, 23-26, 30-31, 33, 35-37 and 45 were rejected under 35 USC § 112, first paragraph, because the specification allegedly doesn't provide enablement for the range of methods claimed. The Examiner states that the specification provides enablement for a treatment composition comprising furosemide or loop diuretic such as furosemide and furosemide-related compositions but does not reasonably provide enablement for numerous specific terms, including "a loop diuretic," "Na⁺K⁺2Cl⁻ cotransporter antagonist activity," etc. This rejection is respectfully traversed in view of the above amendments and the remarks set out below.

Applicant's independent claims have been amended to recite methods for treating migraine headaches and symptoms of migraine headaches (claims 21 and 45), a method for reversing prolonged migraine aura (claim 35), and a method for treating a patient who suffers from migraine headaches and premonitory symptoms of migraine headache (claim 38). Methods for treating cortical spreading depression have been deleted from the claims. The treatment compositions used in the methods include Na⁺K⁺2Cl⁻ cotransporter antagonists (claims 21 and 35), a loop diuretic (claim 38), and a cation chloride cotransporter antagonist (claim 45). Dependent claims further characterize the treatment compound as selected from the group consisting of furosemide, furosemide-related compositions, bumetanide and ethacrynic acid (claim 27), thiazide or a thiazide-like composition (claim 46). Numerous types of delivery methods and formats are claimed.

The Examiner enumerates the Wands factors relevant to enablement, which is determined as of the filing date of the application. The first Wands factor, the nature of the invention, as defined by the pending claims, is methods of treating migraine headaches and (premonitory) symptoms of migraine headaches (claims 21, 38 and 45) and for reversing prolonged migraine aura (claim 35).

The second Wands factor is the state of the art at the time the application was filed. The Examiner states that the art recognizes the treatment of migraine headache, cortical spreading depression and/or the treatment of migraine by controlling "visual aura" via administering furosemide. Applicant *disagrees* that the state of the art at the time of applicant's invention recognized the use of furosemide for *treatment of migraine headache*. The only two references the Examiner cites against applicant's claims relate to treatment of regenerative cortical spreading depression (CSD) in anaesthetized cats with furosemide (Read et al.) and the control of

symptoms of refractory transformed migraine type of chronic daily headache in combination with increased intracranial pressure without papilledema with acetazolamide and furosemide in addition to prophylactic antimigraine medications such as ergotamine, dihydroergotamine (DHE) and sumatriptan (Mathew et al.). These references do *not* recognize the use of furosemide for treatment of migraine headache and symptoms of migraine headache, or for reversing prolonged migraine aura. The state of the art *subsequent to* applicant's invention provides validation for applicant's claimed methods. An article published in September 2000, for example, presents two (human) patients having prolonged migrainous aura who were successfully treated with intravenously administered furosemide. Rozen, *Treatment of a prolonged migrainous aura with intravenous furosemide*, 732 NEUROLOGY 55, September 2000. A copy of this reference is provided with the Information Disclosure Statement, filed herewith.

The third and fourth Wands factors are the relative skill of those in the art and the predictability in the art. Applicant agrees that the relative skill of those in the pharmaceutical art is high and that, *in general*, the unpredictability of the pharmaceutical art is high. It is *generally* true that chemical and biological compounds often react unpredictably under different circumstances. Applicant has, however, identified not a single chemical compound for use in the claimed methods, but a mechanism of action that is put into play by a class of compounds, namely $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists. Because the applicant has identified the underlying mechanism of action it is, in fact, highly predictable that all members of the class, namely $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists, will behave in a similar fashion, although different $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists may exhibit different efficacies. When a mechanism of action has been elucidated and there is substantial data supporting the mechanism of action, as there is in the instant situation, it is urged that it is highly predictable that all members of the identified class will react in a similar fashion and show efficacy, though perhaps to different degrees.

The fifth Wands factor is the breadth of the claims. The Examiner states that the applicant's claims are very broad due to the "vast number of possible compounds that are described as being $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists." The Examiner doesn't give any examples of such compounds or citations to references listing vast numbers of $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists. In fact, the number of such compounds identified to date is quite small. "Loop diuretics" or "High Ceiling" diuretics are, by definition, $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter

antagonists. They act on the tubular epithelial cells in the thick ascending limb of the loop of Henle's to inhibit the $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransport mechanism. The Ninth Edition of *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (1996) describes loop diuretics, or high-ceiling diuretics, as inhibitors of $\text{Na}^+\text{K}^+\text{2Cl}^-$ symport and cites eight examples of $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists, referred to as inhibitors of $\text{Na}^+\text{K}^+\text{2Cl}^-$ symport: Furosemide; Bumetanide; Ethacrynic acid; Torsemide; Azosemide; Musolimine; Piretanide; and Tripamide. Although these compounds are chemically diverse, they are grouped and classified by their functional activity. $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists are found in many secretory and absorbing epithelia. A copy of the Goodman & Gilman section on Inhibitors of $\text{Na}^+\text{K}^+\text{2Cl}^-$ symport (loop diuretics; high-ceiling diuretics) is attached hereto as Exhibit A for the Examiner's reference.

The class of "loop diuretics" is confined to a small number of compounds that have clearly recognized functional activities and does *not* comprehend a vast number of possible compounds. Applicant's claims are not intended or required to be limited to the loop diuretics cited in Goodman & Gilman or any other reference, but are intended to encompass compositions that function as loop diuretics and may therefore encompass analogues or derivatives of known loop diuretics, newly discovered loop diuretics, or the like. This class of compounds is confined and has clearly recognized and easily ascertainable functional activities. It is therefore submitted that applicant's claims directed to methods for treating migraine headache and symptoms, and for reversing prolonged migraine aura by administering a loop diuretic are not overly broad and are fully supported by applicant's specification and experimental evidence.

Applicant's pending claims are directed, independently, to the use of $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists as a class. To applicant's knowledge, there are no $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists identified to date that are not also loop diuretics. It is possible, however, that there are compounds that antagonize the $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter mechanism in the CNS, for example, and not in the loop of Henle in the kidney and these compounds would therefore not be recognized as "loop diuretics." The term $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist is therefore not necessarily coextensive with the term loop diuretic and this class of treatment compositions must be separately claimed.

The applicant has shown that $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists operate in the CNS to desynchronize neuronal population activity, thereby providing effective treatment of conditions

such as seizure disorders, migraine headache and symptoms of migraine headache. Furosemide is the $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist used experimentally by applicant and used by other researchers in subsequent work, largely because it has a well-established safety profile and it shows $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist activity in the CNS. Applicant's elucidation of the mechanism of action involving inhibition of the $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter mechanism provides strong evidence that *all* $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists having activity in the CNS will work in the same manner. Some may be more efficacious than others, but the applicant's experimental work has demonstrated that all compounds having the requisite $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist activity are highly likely to elicit the desired response.

The class of " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists" is confined to a small number of compounds that are easily ascertained and are not necessarily coextensive with the class of claimed loop diuretics. The class of " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists" does *not* comprehend a vast number of possible compounds. It is therefore submitted that applicant's claims, directed to methods for treating migraine headache and symptoms, and for reversing prolonged migraine aura by administering a $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist that is capable of inhibiting $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransport in glial cells are not overly broad and are fully supported by applicant's specification and experimental evidence of the mechanism of action.

The sixth Wands factor relates to guidance in the specification. The Examiner alleges that the specification provides no guidance, in the way of enablement, for claimed agents other than furosemide, more broadly loop diuretics. The Examiner takes the position, again, that numerous possible compounds described by applicant's recitation of "loop diuretics" and " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists" are not necessarily structurally related and it is not obvious from the disclosure of one species what other species will work.

It is submitted that the Examiner's concerns are overstated in the present circumstances. As pointed out above, the classes of both "loop diuretics" and " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists" are confined to well-recognized and easily ascertainable members that are identified on the basis of clear functional activities. There is no confusion about what compounds function as "loop diuretics" and " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists." These compounds are well known in the art. The applicant has elucidated a mechanism of action that underlies the role of "loop diuretics" and " $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists" in treating seizure disorders, migraine and symptoms of migraine, and provides ample experimental evidence supporting that

mechanism of action. Based on this identification of the mechanism of action, it is highly likely, and expected, that all members of the recited classes of compounds will provide effective treatment of migraine and migraine symptoms to some degree, though different degrees of efficacy may be expected. One of ordinary skill in the art would be sufficiently appraised of the compounds suitable for use in applicant's claimed methods and would anticipate, based on the mechanism of action identified, that it is likely that all are suitable for use in applicant's claimed methods.

The seventh and eighth Wands factors are the presence or absence of working examples and the amount of experimentation necessary. The mechanism of action identified by applicant and the application of the mechanism to enumerated therapeutic methods is well supported by applicant's specification, including both the description and experimental evidence provided therein. Experimental results demonstrating treatment of migraine by administering "loop diuretics" and " $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter antagonists" are not provided, but clear guidance concerning methods of administration and the like are provided. At least one subsequent publication demonstrates that a clinician had no difficulty whatsoever with undue experimentation and showed remarkable clinical results, in humans experiencing prolonged migrainous aura, by administering furosemide. See, for example, Rozen, *Treatment of a prolonged migrainous aura with intravenous furosemide*, 732 NEUROLOGY 55, September 2000, a copy of which is provided with the Information Disclosure Statement filed herewith.

It is urged that applicant's pending claims are enabled by the specification and satisfy the requirements of 35 USC § 112. Withdrawal of this rejection is respectfully requested.

Claim Rejections – 35 USC § 102

Read et al.

Claims 21, 23, 25-27, 30-31, 33 and 25-40 are rejected under 35 USC §102(b) as being anticipated by Read et al. (Cephalalgia, 1997, December, 17(8):826-832. This rejection is respectfully traversed.

The subject patent application claims priority to a provisional U.S. patent application filed December 23, 1998; the critical date for § 102(b) prior art is therefore December 23, 1997. Applicant's representative earlier provided evidence that two libraries received their December 1997 issues of Cephalalgia in early January 1998. The Examiner rejected this showing, stating

that according to the information obtained by the Examiner, Cephalalgia is published 8 issues/year, usually in the first week of the month, and delivers to the subscriber. Considering the usual monthly publication date, the dates of communications between authors and editors and the absence of evidence to the contrary, the Examiner maintained his rejection under § 102(b).

A literature publication or magazine article is effective as a printed publication under 35 U.S.C. § 102(b) as of the date it reached the addressee(s) and *not* the date it was placed in the mail. MPEP 706.02(a). Systematic research was conducted by applicant's representative both into the publishing history of Cephalalgia and a more representative sample of recipient institutions. The results of surveying nine (9) additional major institutions in Norway, other Scandinavian countries, Europe, Canada and the United States demonstrate that all recipients received the December 1997 issue of Cephalalgia in January or February 1998. None was received prior to January 5, 1998. The Declaration of Jean Lee, describing the results of her research to ascertain the receipt date of the December 1997 issue of Cephalalgia at institutions in both the U.S. and Europe is attached as Exhibit B. There is consistent evidence that the December issue of Cephalalgia was not received until January 1998 and no evidence that the December issue of Cephalalgia was received by any subscriber on or before the critical date of December 23, 1997.

Even if the Examiner were to assume that Read et al. was available to subscribers or the public prior to applicant's critical date, or if further investigation were to show that selected subscribers *did* receive the December 1997 issue of Cephalalgia prior to the critical date, it is submitted that Read et al. do not anticipate applicant's amended claims, nor would any of the teachings of Read et al., alone or in combination with others, render applicant's claimed methods obvious.

Read et al. presents experimental data showing that furosemide inhibits regenerative cortical spreading depression in anaesthetized cats. The authors hypothesize a mechanism for inhibition of spreading depression in cats by furosemide and speculate that these mechanisms may represent potential novel drug targets in future migraine therapy. Applicant's claims, as amended, are directed to methods for treating migraine headache and premonitory symptoms of migraine headache, and for reversing prolonged migraine aura, all in human subjects. It is submitted that Read et al. is not a proper § 102(b) prior art reference and is, at best, a § 103 prior art reference. The Examiner bears the burden of establishing a *prima facie* case of obviousness

based upon the prior art. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference itself or in the knowledge generally available to one of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. *The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure.* MPEP § 706.02(j).

Read et al. speculate that the mechanism of inhibition of spreading depression d.c. potential activity by furosemide *may* represent potential novel drug targets in future migraine therapy. This speculation is based on their experimental observations that furosemide inhibits regenerative cortical spreading depression in anaesthetized cats. Applicant agrees that it may be obvious to try furosemide treatment of migraine based on Read et al.'s speculation. Applicant submits, however, that there would be no reasonable expectation of success based on the teachings of Read et al. and the state of art at the time the application was filed. It was not until applicant's elucidation of the role of $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists in modulating (reducing) the synchronization of neuronal population activity that is associated with seizure disorders and migraine headaches, and other pathophysiologies of the central nervous system, that one of ordinary skill in the art would have had any reasonable expectation that $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists and loop diuretics would be effective in treating migraine headaches and symptoms.

Cortical spreading depression has been observed in various animals, including rabbits, rats and cats. Whether humans experience cortical spreading depression, or whether a phenomenon that is the same as or similar to the cortical spreading depression as observed in animals precedes migraine headache in humans is, as far as applicant is aware, unknown and has been the subject of much scientific debate. Researchers at the same research facility as Read et al. concluded, in abstract for a paper published in May 2001:

“The possibility that spreading depression (SD) of cortical activity, a phenomenon observed in all vertebrates, causes the aura of migraine remains an open question in spite of nearly half a century of investigation.” M.F. James et al., *Cortical spreading depression and migraine: new insights from imaging?*, Trends Neurosci. 2001 May;24(5):266-71.

Experimental results intended to demonstrate a link between cortical spreading depression and nociceptive activity and other physiological changes that are believed to produce migraine headaches using a rat model were also published in 2001. The results did *not* demonstrate a link between cortical spreading depression and migraine. The authors observe, at page 12:

A final question concerns the relevance of the present findings to human migraine. Although some studies have provided evidence, based on blood flow measurements and functional MRI studies, that CSD exists in humans, others have failed to reveal changes before the onset of headache. It has also been shown that classical antimigraine drugs, such as sumatriptan or vasodilator agents, failed to affect CSD initiation or propagation in the cat. These findings also raise the question of whether CSD is important for the initiation of nociception. Ebersberger et al., *Is There a Correlation Between Spreading Depression, Neurogenic Inflammation, and Nociception That Might Cause Migraine Headache?* Annals of Neurology Vol. 49, No. 1, January 2001.

In yet another, very recent publication authored by one of the co-authors of the Read et al. reference cited against applicant's claims, the role of cortical spreading depression in migraine pathogenesis is discussed. The author concludes:

Considering all of the evidence, it appears that a spreading neurovascular event very similar to CSD in animal models occurs during migraine aura. However, the functional importance of this process still needs to be elucidated. One also may agree that there is some direct evidence linking CSD to only a few patients with migraine, although there is considerable evidence for some cortical dysfunction in migraine. The hypothesis that CSD plays a role in these disturbances still remains to be tested. A.A. Parsons, *Cortical Spreading Depression: Its Role in Migraine Pathogenesis and Possible Therapeutic Intervention Strategies*, Current Pain and Headache Reports 2004, 8:410-416.

Copies of these references are attached hereto as Exhibit C for the Examiner's reference.

Based on the controversy and differing views in the art as to whether cortical spreading depression has any relationship to migraine headaches, and whether cortical spreading depression is even observed in humans, applicant submits that one of ordinary skill in the art, at the time the invention was made, *may* have been motivated to follow the suggestion of Read et al. but would *not* have had a reasonable expectation that administering a loop diuretic or $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist to humans would successfully treat migraine headaches or symptoms, or reverse prolonged migraine aura. Applicant's elucidation of the role of $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists in modulating (reducing) the synchronization of neuronal population activity

associated with seizure disorders and migraine headaches would provide a reasonable expectation of success, but the subject matter of the pending application cannot be considered. It is therefore urged that applicant's pending claims are not obvious in view of Read et al.

Mathew et al.

Claims 21, 23-24, 27-32 and 35-38 were rejected under 35 U.S.C. § 102(b) as being anticipated by Mathew et al, Neurology, 1996; 46:1226-1230. This rejection is respectfully traversed, particularly in view of the above amendments and the following remarks.

Mathew et al. studied patients with refractory transformed migraine type of chronic daily headache (CDH), and showed that a *subset* of these patients who were treated after diagnosis of increased intracranial pressure with a combination of antimigraine agents (including ergotamine, sumatriptan and dihydroergotamine (DHE)), acetazolamide and furosemide *to reduce increased intracranial pressure*, showed reduced number of days of severe headache, reduced consumption of abortive agents and overall improvement of quality of life. Prophylactic agents such as beta blockers, tricyclic antidepressants, calcium channel blockers and serotonin antagonists were used for treatment of chronic daily headache. Nonsteroidal anti-inflammatory agents were also used. The authors concluded that any patient with chronic daily headache with migrainous features who is refractory to conventional therapy with prophylactic antimigraine agents should have a spinal tap to exclude coexisting idiopathic intracranial hypertension (IIH). The authors further speculate that there may be a link or shared pathophysiology between IIH and migraine. The Examiner argues that the applicant's claims don't exclude combination therapy and that the use of furosemide in the combination therapies of Mathews et al. anticipates the claimed methods.

Mathew et al. focused on and administered combination therapy to a subset of refractory transformed migraine type of chronic daily headache having elevated CSF pressure as measured by a lumbar puncture. The comparison in patient populations studied by Mathew et al. is CDH patients with and without IIH. In CDH patients with normal intracranial pressure, the response to migraine prophylactic agents alone was good. In patients with CDH and IIH, the addition of acetazolamide and furosemide further improved overall headache control. It is submitted that Mathew et al., at best, suggests that the combination of acetazolamide and furosemide with conventional anti-migraine treatments may be effective in patients having *both* refractory transformed migraine type of chronic daily headache *and* having elevated CSF pressure.

The use of acetazolamide and furosemide in combination with numerous other migraine prophylactic agents, it is submitted, does *not* anticipate applicant's pending claims directed to methods for treating migraine headaches and symptoms, and of reversing prolonged migraine aura, by administering either loop diuretics or $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonists. Furthermore, even if one of ordinary skill in the art may have been motivated to try treating migraine headaches and symptoms with a combination of acetazolamide and furosemide, or with one of these agents as a consequence of Read et al., there would be no reasonable expectation of success without knowledge of applicant's work described in the pending patent application. It is therefore urged that Mathew et al. do not render applicant's claims obvious in the manner required by 35 U.S.C. § 103.

Claim Rejections – 35 USC § 103

Claims 41-44 were rejected under 35 U.S.C. § 103 as being unpatentable over Read et al. (Cephalalgia) or Mathew et al. (Neurology). This rejection is respectfully traversed, particularly in view of the above amendments and the following remarks.

Claims 41-44 and several newly added claims recite subject matter relating to specific methods of administration, such as intranasal administration (claims 41 and 42), administration directly to the cerebrospinal fluid (claims 43 and 44), transdermal delivery for delivery to the CNS (claim 47), as a sustained release formulation (claim 48), in a dosage incorporated in a non-reactive carrier (claim 49), in a liposome formulation (claim 50), by implantation of a formulation or therapeutic device at one or more target sites for delivery of the treatment composition to the CNS (claims 51 and 52), and administration in combination with a hyperosmotic agent (claim 53).

To establish a *prima facie* case of obviousness, the Examiner must fulfill three requirements. First, there must be some suggestion or motivation, either in the reference itself or in the knowledge generally available to one of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. The deficiencies of the Read et al. and Mathew et al. references with respect to applicant's independent claims are described above. One of ordinary skill in the art would *not* have been motivated to treat migraine, migraine symptoms and prolonged migraine aura by administering a loop diuretic or $\text{Na}^+\text{K}^+\text{2Cl}^-$ cotransporter antagonist in

view of the teachings of Read et al. and Mathew et al. and would certainly not have found a reasonable expectation of success in such a treatment based on the teachings of either Read et al. or Mathew et al.

With respect to the specific delivery forms claimed by applicant, applicant submits there is no suggestion or motivation whatsoever found in either of the references relied upon by the Examiner, or in the knowledge generally available to one of ordinary skill in the art, to modify the teachings of the references to include applicant's claimed delivery forms and regimen. There is no indication in the prior art that any particular delivery regimen is required, or desirable, for delivery of treatment compositions for treatment of either cortical spreading depression in anaesthetized cats or the combination of chronic daily headache and idiopathic intracranial hypertension in humans. There is no suggestion or motivation to use the delivery formats claimed by applicant, namely: intranasal delivery, delivery directly into the cerebrospinal fluid, transdermal delivery to the CNS, delivery in a sustained release formulation, delivery in a dosage incorporated in a non-reactive carrier or liposome formulation, or delivery via implantation of a formulation or therapeutic device for delivery of the treatment agent to the CNS. The prior art relied upon for rejection does *not* teach or suggest these claim features. Although these delivery forms and regimen are not unique in and of themselves and various delivery forms and regimen have been used in connection with other therapeutic compositions, there is no indication that, when combined with applicant's claimed methods and the treatment compositions used in applicant's claimed methods, these delivery forms and regimen would be obvious to one of ordinary skill in the art.

It is urged that applicant's dependent claims would not be obvious to one of ordinary skill in the art in the manner required by 35 U.S.C. § 103. Withdrawal of this rejection is respectfully requested.

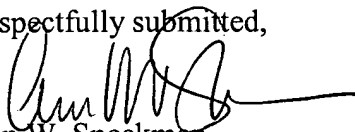
Conclusion

In view of the above amendments and remarks, it is believed that the applicant has successfully overcome the outstanding claim rejections, and that the claims now pending are in condition for allowance. If the Examiner has any further comments or questions, he is invited to contact the undersigned representative for the applicant.

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Please charge any additional fees that may be required, or credit any overpayment, to our Deposit Account No. 19-3555.

Respectfully submitted,



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Registration No. 31,881

Date: November 24, 2004

SPECKMAN LAW GROUP PLLC
20601

GOODMAN & GILMAN's The PHARMACOLOGICAL BASIS OF THERAPEUTICS

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sure during acute attacks of glaucoma and for short-term reductions in intraocular pressure, both preoperatively and postoperatively, in patients who require ocular surgery. Also, mannitol and urea are used to reduce cerebral edema and brain mass before and after neurosurgery.

INHIBITORS OF $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ SYMPORT (LOOP DIURETICS; HIGH-CEILING DIURETICS)

Inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport are a group of diuretics that have in common an ability to block the $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symporter in the thick ascending limb of the loop of Henle; hence these diuretics also are referred to as *loop diuretics*. Although the proximal tubule reabsorbs approximately 65% of the ultrafiltrate, diuretics acting only in the proximal tubule have limited efficacy because the thick ascending limb has a great reabsorptive capacity and reabsorbs most of the rejectate from the proximal tubule. Diuretics acting predominantly at sites past the thick ascending limb also have limited efficacy because only a small percentage of the filtered load ever reaches these more distal sites. In contrast, inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport are highly efficacious, and for this reason they often are called *high-ceiling* diuretics. The efficacy of inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport in the thick ascending limb of the loop of Henle is due to a combination of two factors: (1) approximately 25% of the filtered solute load normally is reabsorbed by the thick ascending limb, and (2) nephron segments past the thick ascending limb do not possess the reabsorptive capacity to rescue the flood of rejectate exiting the thick ascending limb.

Chemistry. Inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport are a chemically diverse group of drugs (see Table 29-4). Furosemide, bumetanide, azosemide, piretanide, and triamamide all contain a sulfonamide moiety, whereas ethacrynic acid is a phenoxyacetic acid derivative. Muzolimine has neither of these structural features, and torsemide is a sulfonylurea. Only *furosemide* (LASIX), *bumetanide* (BUMEX), *ethacrynic acid* (EDECRIN), and *torsemide* (DEMADEX) are available currently in the United States.

Mechanism and Site of Action. Overwhelming evidence indicates that inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport act primarily in the thick ascending limb. Micropuncture of the DCT demonstrates that loop diuretics increase the delivery of solutes out of the loop of Henle (Dirks and Seely, 1970). Also, *in situ* microperfusion of the loop of

Henle (Morgan *et al.*, 1970) and *in vitro* microperfusion of the CTAL (Burg *et al.*, 1973) indicate inhibition of transport by low concentrations of furosemide in the perfusate. Some inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport, particularly furosemide, may have additional effects in the proximal tubule; however, the significance of these effects is unclear.

It was initially thought that Cl^- was transported by a primary active electrogenic transporter in the luminal membrane independent of Na^+ . Discovery of furosemide-sensitive $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport in other tissues caused Greger (1981) to investigate more carefully the Na^+ dependence of Cl^- transport in the isolated perfused rabbit CTAL. By scrupulously removing Na^+ from the luminal perfusate, Greger demonstrated the dependence of Cl^- transport on Na^+ . It is now well accepted that, in the thick ascending limb, flux of Na^+ , K^+ , and Cl^- from the lumen into the epithelial cell is mediated by a $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symporter (see Figure 29-6). This symporter captures the energy in the Na^+ electrochemical gradient established by the basolateral Na^+ pump and provides for "uphill" transport of K^+ and Cl^- into the cell. K^+ channels in the luminal membrane provide a conductive pathway for the apical recycling of this cation, and basolateral Cl^- channels provide a basolateral exit mechanism for Cl^- . In addition, a $\text{Na}^+ - \text{Cl}^-$ symporter in the basolateral membrane permits cotransport of Cl^- down an electrochemical gradient with concomitant transport of Na^+ against an electrochemical gradient. The luminal membranes of epithelial cells in the thick ascending limb have conductive pathways (channels) only for K^+ , and therefore the apical membrane voltage is determined by the equilibrium potential for K^+ (E_K). In contrast, the basolateral membrane has channels for both K^+ and Cl^- , so that the basolateral membrane voltage is less than E_K , i.e., conductance for Cl^- depolarizes the basolateral membrane. Depolarization of the basolateral membrane results in a transepithelial potential difference of approximately 10 mV, with the lumen positive with respect to the interstitial space. This lumen-positive potential difference repels cations (Na^+ , Ca^{2+} , and Mg^{2+}) and thereby provides an important driving force for the paracellular flux of these cations into the interstitial space.

As the name implies, inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport bind to the $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symporter in the thick ascending limb (Koenig *et al.*, 1983) and block its function, bringing salt transport in this segment of the nephron to a virtual standstill (Burg *et al.*, 1973). The molecular mechanism by which this class of drugs blocks the $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symporter is unknown, but evidence suggests that these drugs attach to the Cl^- -binding site of the symporter (Hannafin *et al.*, 1983). Inhibitors of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport also inhibit Ca^{2+} and Mg^{2+} re-

Table 29-4

Inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ Symport (Loop Diuretics; High-Ceiling Diuretics)

DRUG	STRUCTURE	RELATIVE POTENCY	ORAL ABSORPTION	$t_{1/2}$	ROUTE OF ELIMINATION
Furosemide		1	11-90%	0.3-3.4 h	60% R, 40% M
Bumetanide		40	59-89%	0.3-1.5 h	65% R, 35% M
Ethacrynic acid		0.7	Nearly complete	0.5-1 h	65% R, 35% M
Torsemide		3	79-91%	0.8-6.0 h	30% R, 70% M
Azosemide*		ID	ID	ID	ID
Muzolimine*		ID	ID	ID	ID
Piretanide*		3	~80%	0.6-1.5 h	50% R, 50% M
Tripamide*		ID	ID	ID	ID

*Not available in the United States.

Abbreviations: R, renal excretion of intact drug; M, metabolism; ID, insufficient data.

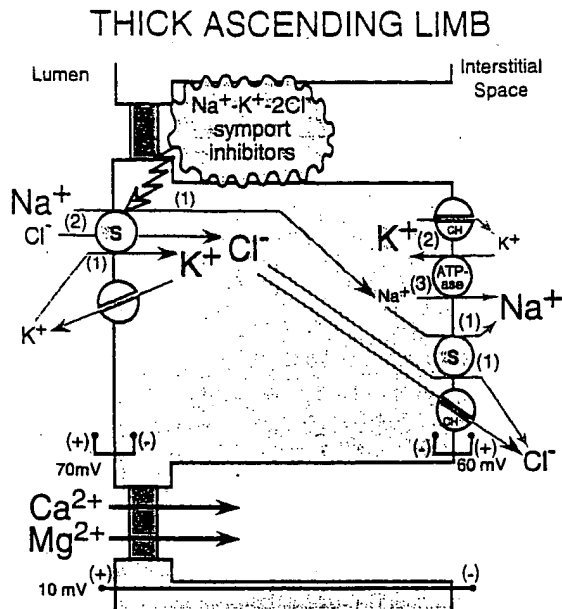


Figure 29-6. NaCl reabsorption in thick ascending limb and mechanism of diuretic action of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport inhibitors.

S, symporter; CH, ion channel. Numbers in parentheses indicate stoichiometry. Designated voltages are the potential differences across the indicated membrane or cell.

absorption in the thick ascending limb by abolishing the transepithelial potential difference that is the dominant driving force for reabsorption of these cations.

$\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporters are an important family of transport molecules found in many secretory and absorbing epithelia. In particular, the rectal gland of the dogfish shark is one of the richest sources of the protein, and a cDNA encoding a $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter was isolated from a cDNA library obtained from the dogfish shark rectal gland by screening with monoclonal antibodies to the shark symporter (Xu *et al.*, 1994). Molecular cloning revealed a deduced amino acid sequence of 1191 residues containing 12 putative membrane-spanning domains flanked by long N and C termini in the cytoplasm. Expression of this protein in HEK-293 cells resulted in $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport that was sensitive to bumetanide. The shark rectal gland $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter cDNA subsequently was used to screen a human colonic cDNA library, and this provided $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter cDNA probes from this tissue. These latter probes were used to screen rabbit renal cortical and renal medullary libraries, which allowed cloning of the rabbit renal $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter (Payne and Forbush, 1994). This symporter is 1099 amino acids in length, is 61% identical to the dogfish shark secretory $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter, has 12 predicted transmembrane helices, contains large N- and C-terminal cytoplasmic regions, and has 3 potential sites for phosphorylation by protein kinase A in its C terminus. Of potential physiological importance, three splice variants of the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter have been discovered, one expressed only in the cortex, one expressed only in the medulla, and one expressed in both regions. Gamba *et al.* (1994) also recently have cloned a kidney $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter

from a rat. In the near future, the binding site(s) for loop diuretics on the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter probably will be elucidated, leading to greater knowledge of the mechanism of action of the symporter. This information may provide new strategies for pharmacological manipulation of this important transporter.

Effects on Urinary Excretion. Due to blockade of the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symporter, loop diuretics cause a profound increase in the urinary excretion of Na^+ and Cl^- (i.e., up to 25% of the filtered load of Na^+). Abolition of the transepithelial potential difference also results in marked increases in the excretion of Ca^{2+} and Mg^{2+} . Some (e.g., furosemide), but not all (e.g., bumetanide and piretanide), sulfonamide-based loop diuretics have weak carbonic anhydrase-inhibiting activity. Those drugs with carbonic anhydrase-inhibiting activity increase the urinary excretion of HCO_3^- and phosphate. The mechanism by which inhibition of carbonic anhydrase increases phosphate excretion is not known. All inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport increase the urinary excretion of K^+ and titratable acid. This effect is due in part to increased delivery of Na^+ to the distal tubule. The mechanism by which increased distal delivery of Na^+ enhances excretion of K^+ and H^+ is discussed in the section on inhibitors of Na^+ channels. Acutely, loop diuretics increase the excretion of uric acid, whereas chronic administration of these drugs results in reduced excretion of uric acid. The chronic effects of loop diuretics on uric acid excretion may be due to enhanced transport in the proximal tubule secondary to volume depletion, leading to increased uric acid reabsorption, or to competition between the diuretic and uric acid for the organic acid secretory mechanism in the proximal tubule, leading to reduced uric acid secretion.

By blocking active NaCl reabsorption in the thick ascending limb, inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport interfere with a critical step in the mechanism that produces a hypertonic medullary interstitium. Therefore, loop diuretics block the kidney's ability to concentrate urine during hyponatremia. Also, since the thick ascending limb is part of the diluting segment, inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport markedly impair the kidney's ability to excrete a dilute urine during water diuresis.

Effects on Renal Hemodynamics. If volume depletion is prevented by replacing fluid losses, inhibitors of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ symport generally increase total RBF and redistribute RBF to the midcortex (Stein *et al.*, 1972). However, the effects on RBF are notoriously variable, increasing in many studies and not changing in many others. The mechanism of the increase in RBF is not known, but prostaglandins have been implicated (Williamson *et al.*,

1974). In fact, nonsteroidal antiinflammatory drugs (NSAIDs) attenuate the diuretic response to loop diuretics, most likely by preventing prostaglandin-mediated increases in RBF (Brater, 1985). Loop diuretics block TGF, probably by inhibiting salt transport into the macula densa, so that the macula densa can no longer "sense" NaCl concentrations in the tubular fluid. Therefore, unlike carbonic anhydrase inhibitors, loop diuretics do not decrease GFR by activating TGF. Loop diuretics are powerful stimulators of renin release. This effect is due to interference with NaCl transport by the macula densa and, if volume depletion occurs, to reflex activation of the sympathetic nervous system and to stimulation of the intrarenal baroreceptor mechanism. Prostaglandins, particularly prostacyclin, may play an important role in mediating the renin release response to loop diuretics (Oates *et al.*, 1979).

Other Actions. Loop diuretics, particularly furosemide, acutely increase systemic venous capacitance and thereby decrease left ventricular filling pressure. This effect, which may be mediated by prostaglandins and requires intact kidneys (Johnston *et al.*, 1983), benefits patients with pulmonary edema even before diuresis ensues. Furosemide and ethacrynic acid can inhibit Na^+ , K^+ -ATPase, glycolysis, mitochondrial respiration, the microsomal Ca^{2+} pump, adenylyl cyclase, phosphodiesterase, and prostaglandin dehydrogenase; however, these effects do not have therapeutic implications. *In vitro*, high doses of inhibitors of Na^+ - K^+ - 2Cl^- symport can inhibit electrolyte transport in many tissues. Only in the inner ear, where alterations in the electrolyte composition of endolymph may contribute to drug-induced ototoxicity, is this effect clinically important.

Absorption and Elimination. The oral bioavailability, plasma half-life, and route of elimination of the four inhibitors of Na^+ - K^+ - 2Cl^- symport available in the United States are listed in Table 29-4. Because furosemide, bumetanide, ethacrynic acid, and torsemide are extensively bound to plasma proteins, delivery of these drugs to the tubules by filtration is limited. However, they are efficiently secreted by the organic acid transport system in the proximal tubule and thereby gain access to their binding sites on the Na^+ - K^+ - 2Cl^- symport in the luminal membrane of the thick ascending limb. Probenecid shifts the plasma concentration-response curve to furosemide to the right by competitively inhibiting furosemide secretion by the organic acid transport system (Brater, 1983). The most recent loop diuretic to receive FDA approval is torsemide, which has a longer half-life than the other loop diuretics that are available in the United States (Brater, 1991).

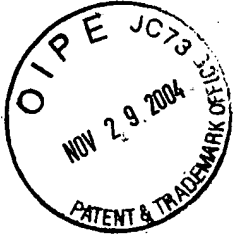
Toxicity, Adverse Effects, Contraindications, Drug Interactions. Adverse effects unrelated to the diuretic efficacy are rare, and most adverse effects are due to abnormalities of fluid and electrolyte balance. Overzealous use of loop diuretics can cause serious depletion of total body Na^+ . This may manifest as hyponatremia and/or extracellular fluid volume depletion associated with hypotension, reduced GFR, circulatory collapse, thromboembolic episodes, and, in patients with liver disease, hepatic encephalopathy. Increased delivery of Na^+ to the distal tubule, particularly when combined with activation of the renin-angiotensin system, leads to increased urinary excretion of K^+ and H^+ , causing a hypochloremic alkalosis. If dietary K^+ intake is not sufficient, hypokalemia may develop, and this may induce cardiac arrhythmias, particularly in patients taking cardiac glycosides. Increased Mg^{2+} and Ca^{2+} excretion may result in hypomagnesemia (a risk factor for cardiac arrhythmias) and hypocalcemia (rarely leading to tetany).

Loop diuretics can cause ototoxicity that manifests as tinnitus, hearing impairment, deafness, vertigo, and a sense of fullness in the ears. Hearing impairment and deafness are usually, but not always, reversible. Ototoxicity occurs most frequently with rapid intravenous administration and least frequently with oral administration. Ethacrynic acid appears to induce ototoxicity more often than do other loop diuretics. Loop diuretics also can cause hyperuricemia (rarely leading to gout) and hyperglycemia (rarely precipitating diabetes mellitus) and can increase plasma levels of LDL cholesterol and triglycerides, while decreasing plasma levels of HDL cholesterol. Other adverse effects include skin rashes, photosensitivity, paresthesias, bone marrow depression, and gastrointestinal disturbances.

Contraindications for loop diuretics include severe Na^+ and volume depletion, hypersensitivity to sulfonamides (for sulfonamide-based loop diuretics), and anuria unresponsive to a trial dose of loop diuretic.

Drug interactions may occur when loop diuretics are coadministered with: (1) aminoglycosides (synergism of ototoxicity caused by both drugs); (2) anticoagulants (increased anticoagulant activity); (3) digitalis glycosides (increased digitalis-induced arrhythmias); (4) lithium (increased plasma levels of lithium); (5) propranolol (increased plasma levels of propranolol); (6) sulfonylureas (hyperglycemia); (7) cisplatin (increased risk of diuretic-induced ototoxicity); (8) NSAIDs (blunted diuretic response); (9) probenecid (blunted diuretic response); and (10) thiazide diuretics (synergism of diuretic activity of both drugs leading to profound diuresis).

Therapeutic Uses. A major use of loop diuretics is in the treatment of acute pulmonary edema. A rapid increase in venous capacitance in conjunction with a brisk natriuresis reduces left ventricular filling pressures and thereby rapidly relieves pulmonary edema. Loop diuretics also are widely used for the treatment of chronic congestive heart failure when diminution of extracellular fluid volume is desirable to minimize venous and pulmonary congestion (*see* Chapter 34). Diuretics are widely used for the treatment of hypertension (*see* Chapter 33); however, in this regard, loop diuretics are not the diuretic of first choice and are reserved for patients in whom other diuretics or antihypertensive drugs do not result in a satisfactory response. The edema of nephrotic syndrome often is refractory to other classes of diuretics, and loop diuretics often are the only drugs capable of reducing the massive edema associated with this renal disease. Loop diuretics also are employed in the treatment of edema and ascites of liver cirrhosis; however, care must be taken not to induce encephalopathy or hepatorenal syndrome. In patients with a drug overdose, loop diuretics can be used to induce a forced diuresis to facilitate more rapid renal elimination of the offending drug. Loop diuretics combined with isotonic saline administration to prevent volume depletion are used to treat hypercalcemia. Loop diuretics interfere with the kidney's ability to produce a concentrated urine. Consequently, loop diuretics combined with hypertonic saline are useful for the treatment of life-threatening hyponatremia. Loop diuretics also are used to treat edema associated with chronic renal insufficiency. Most patients with ARF receive a trial dose of a loop diuretic in an attempt to convert oliguric ARF to nonoliguric ARF.



CERTIFICATE OF MAILING

I hereby certify that this paper or fee is being deposited with the United States Postal Service with sufficient postage as first class mail under 37 C.F.R. 1.8 on the date indicated below and is addressed to: **MAILSTOP: RCE**, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date: November 24, 2004

Selena Whitaker-Paquet

Attorney Docket No.: 48000.1003c2u
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of **Daryl W. Hochman**

Group Art Unit: 1614

Application No. : 10/056,528
Filed : January 23, 2002
For : **METHODS AND COMPOSITIONS FOR TREATING
CONDITIONS OF THE CENTRAL AND PERIPHERAL
NERVOUS SYSTEMS USING NON-SYNAPTIC MECHANISMS**
Examiner : Brian S. Kwon

DECLARATION OF JEAN LEE

The undersigned, Jean Lee, hereby declares:

1. I am a research and office assistant employed by Speckman Law Group PLLC. I have been so employed since June 2004. I graduated from the University of Washington in June 2004 with a degree in Anthropology and Linguistics.
2. I was assigned the project of determining when the December 1997 issue of *Cephalalgia* (Volume 17, Number 8) was available to the public. Earlier evidence showed that the December 1997 issue of *Cephalalgia* was not received by the Duke Medical Library until January 6, 1998 and was not received by the Thomas Jefferson University Library until January 6, 1998. Evidence of those receipt dates was provided to the Examiner in an earlier filing by applicant's representative.
3. The publisher of the December 1997 issue of *Cephalalgia* was Scandinavian University Press (currently Universitetsforlaget), based in Oslo, Norway. An internet search for "Cephalalgia," however, indicated that the current publisher of the journal is Blackwell Publishing.

4. I made several phone calls to Blackwell Publishing, the current publisher of the journal, and was directed to correspond with Rowena Roche, the production editor for Blackwell in Edinburgh, Scotland. In my e-mail correspondences with Ms. Roche, dated October 20 and October 21, 2004, I inquired of the distribution and/or publication date of the December 1997 issue of *Cephalalgia*. Ms. Roche indicated that she would be unable to provide any information relating to the production and distribution schedule of the issue in question, as Blackwell Publishing does not possess records for issues prior to its acquisition of *Cephalalgia* in 2000. Copies of my e-mail correspondence with Ms. Roche are attached as Exhibit B1.
5. Further research indicated that Scandinavian University Press, the publisher of the 1997 issue of *Cephalalgia*, merged with Tano Aschehoug in March 2000 to form Universitetsforlaget, which subsequently merged with two other publishing houses in February 2004 to form Aschehoug Agency. My October 20th e-mail inquiry to Aschehoug Agency regarding the December 1997 distribution schedule for *Cephalalgia* was answered by Lars Alldén, the head of the Journals department at the time of the 2000 merger. Mr. Alldén indicated that as a result of the merger, the International Journals Division, including *Cephalalgia*, was acquired by Taylor and Francis, UK, and essential files for these journals were moved to a remote archive outside of Oslo. Furthermore, Mr. Alldén noted that there are no extant files that would give any indication of the date the December 1997 issue was mailed to subscribers. He also confirmed that at that time, there would have been no simultaneous publication of the issue in digital form: "In the end of the nineties, the articles were with some delay stored electronically in databases and delivered to customers around the world through third parties," and thus, "the date of publication should be counted as the day of the paper version" (personal e-mail correspondence with Lars Alldén, 21 Oct. 2004). Copies of this e-mail correspondence with Mr. Alldén are attached as Exhibit B2.
6. I obtained from the University of Oslo Library of Medicine and Health a date-stamped copy of the *Read et al.* (*Cephalalgia* 17(8): December 1997) reference cited in the Examiner's Office Action. The date-stamp on this photocopy indicates a library receipt date of January 13, 1998. A copy of the cover page bearing the date stamp is attached as Exhibit B3.


7. Additionally, the University of Oslo library is a member of BIBSYS, a database that catalogs the holdings of the Norwegian National Library, all Norwegian university and college libraries, and a number of Norwegian research libraries. The BIBSYS database contains records of receipt dates for issues of *Cephalalgia* received by BIBSYS member libraries. BIBSYS Record 822122316, listing holdings of *Cephalalgia* for BIBSYS member libraries, is accessible at the url <http://wgate.bibsys.no/gate1/EKSL?objd=822122316&lang=E>. A copy of this web page, listing BIBSYS member libraries with holdings of *Cephalalgia*, is attached as part of Exhibit D. Of the ten BIBSYS libraries that possess holdings for *Cephalalgia*, nine libraries have records of receipt dates available on the BIBSYS database. Of these nine libraries, four subscribed to *Cephalalgia* in December 1997. BIBSYS Record 94f000228, indicating January 9, 1998, as the receipt date of this issue by the Norwegian University of Science and Technology, is accessible at the url <http://wgate.bibsys.no/gate1/EKSL?objd=94f000228&lang=E>. BIBSYS Record 88d007647, indicating February 24, 1998, as the receipt date of this issue by the University of Bergen Medical Library, is accessible at the url <http://wgate.bibsys.no/gate1/EKSL?objd=88d007647&lang=E>. BIBSYS Record 96za00428, indicating January 8, 1998, as the receipt date of this issue by the University of Oslo Library of Medicine and Health, is accessible at the url <http://wgate.bibsys.no/gate1/EKSL?objd=96za00428&lang=E>. It is unknown why the date listed in BIBSYS differs from the January 13, 1998, date stamp on the photocopy of the cover page supplied by the University of Oslo Library of Medicine and Health. BIBSYS Record 93sa13197, indicating February 4, 1998, as the receipt date of this issue by the National Library of Norway, is accessible at <http://wgate.bibsys.no/gate1/EKSL?objd=93sa13197&lang=E>. Copies of all of these web pages, indicating the receipt dates of the December 1997 issue of *Cephalalgia* by four BIBSYS libraries, are also attached as parts of Exhibit B4.
8. I corresponded via e-mail with a number of national and university libraries, inquiring about the libraries' respective receipt dates for the December 1997 issue of *Cephalalgia*. My e-mail correspondence with Marianne Siener of the University of Massachusetts Medical School Library, indicating January 5, 1998, as the date of receipt at this library, is attached as Exhibit B5. E-mail correspondence with Hélène Gautier Gentès, indicating

January 6, 1998, as the date of receipt of this issue by the Bibliothèque Interuniversitaire de Medicine, is attached as Exhibit B6. E-mail correspondences with Liselotte Jørgensen and Per Morten Sørensen, both indicating January 9, 1998 as the date of receipt by the Danish National Library of Science and Medicine, are attached as Exhibit B7. E-mail correspondence with the reference division of the UCLA Biomedical Library, indicating that this library received the December 1997 issue of *Cephalalgia* on January 9, 1998, is attached as Exhibit B8. E-mail correspondence with Manon Cockwell, indicating January 13, 1998, as the receipt date of this issue by the Canada Institute for Scientific and Technical Information, is attached as Exhibit B9.

9. Receipt dates of the December 1997 issue of *Cephalalgia* for the abovementioned libraries, as well as methods of confirmation, are summarized as follows:

Library	Receipt Date	Confirmation Method
University of Massachusetts Medical School Library	January 5, 1998	Correspondence with Marianne Siener: Marianne.Brophy@umassmed.edu
Bibliothèque Interuniversitaire de Médecine	January 6, 1998	Correspondence with Héléne Gautier Gentès: gautierh@bium.univ-paris5.fr
University of Oslo Library of Medicine and Health	January 8, 1998 January 13, 1998	BIBSYS http://wgate.bibsys.no/gate1/EKSL?objd=96za00428&lang=E Date-stamped photocopy of cover page
Norwegian University of Science and Technology	January 9, 1998	BIBSYS http://wgate.bibsys.no/gate1/EKSL?objd=94f000228&lang=E
University of California, Los Angeles Biomedical Library	January 9, 1998	Correspondence with Reference Division of library bio_pc@library.ucla.edu
Danish National Library of Science and Medicine	January 9, 1998	Correspondence with Liselotte Jørgensen lij@DNLB.DK and Per Morten Sørensen PMS@DNLB.DK
Canada Institute for Scientific and Technical Information	January 13, 1998	Correspondence with Manon Cockwell Manon.Cockwell@nrc-cnrc.gc.ca
National Library of Norway	February 4, 1998	BIBSYS http://wgate.bibsys.no/gate1/EKSL?objd=93sa13197&lang=E
University of Bergen	February 24, 1998	BIBSYS http://wgate.bibsys.no/gate1/EKSL?objd=88d007647&lang=E

10. All libraries with which I corresponded and that provided information regarding the receipt date of *Cephalalgia* affirmed that their institutions' records indicate the December 1997 issue of the journal was not received prior to 1998. In the course of my research, I encountered no information evidencing that any national, academic, or research library received the December 1997 issue of *Cephalalgia* prior to 1998.
11. I, the undersigned, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.


Jean Lee

11/24/2004
Date

Date: November 24, 2004

SPECKMAN LAW GROUP

20601

EXHIBIT B1

E-mail correspondence with Rowena Roche

Jean Lee

From: Roche Rowena [Rowena.Roche@edn.blackwellpublishing.com]
Sent: Thursday, October 21, 2004 12:49 AM
To: Jean Lee
Cc: Ann Speckman
Subject: RE: Cephalalgia distribution date

Dear Jean,

Thank you for your message.

I have checked our records and unfortunately, I am unable to help with your query as Blackwell Publishing did not start to publish *Cephalalgia* until 2000. However, I can tell you that the issue in question was published by Scandinavian University Press.

Best wishes,

Rowena

-----Original Message-----

From: Jean Lee [mailto:jeanl@speckmanlaw.com]
Sent: 20 October 2004 19:56
To: Roche Rowena
Cc: Ann Speckman
Subject: Cephalalgia distribution date

Dear Ms. Roche,

Faye Cheeseman at Blackwell Publishing recommended that I contact you regarding the distribution date of a previous issue of *Cephalalgia*. The issue of interest to me is the December 1997 issue of *Cephalalgia* (Volume 7, Number 8), and my particular concern is the earliest date the issue became publicly available anywhere in the world, in either print or electronic form. Date-stamped photocopies of this issue received from Duke University Medical Library and Thomas Jefferson University Library both indicate a library receipt date of January 6, 1998. I would, however, like to confirm with the actual distribution date and/or publication date, if the distribution date is unavailable.

I realize that, because this is a somewhat dated issue, such information may be impossible to obtain. Nevertheless, any assistance on this matter would be greatly appreciated. Thank you.

Best Regards,

Jean Lee, General Assistant
Speckman Law Group PLLC
1501 Western Ave., Suite 100
Seattle, WA 98101
Tel. 206.382.1191
Fax. 206.382.2669
jeanl@speckmanlaw.com

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EXHIBIT B2

E-mail correspondence with Lars Alldén

Jean Lee

From: Lars Alldén [Lars.Alden@aschehoug.no]

Sent: Thursday, October 21, 2004 1:09 AM

To: Jean Lee

Subject: RE: 1997 Universitetsforlaget publication

Dear Jean Lee,

As of March 1st, 2000 Universitetsforlaget (formerly also called Scandinavian University Press) was radically reorganized, and the important parts of the activities were taken over by two other Norwegian publishers, Aschehoug and Gyldendal. The International Journals Division was acquired by Taylor and Francis, UK. For journals which the University Press at that time had ceased to publish, essential files were stored in a remote archive some miles outside Oslo. I was the leader of the Journals department at that time, I am thus able to inform you that no files exist from which it should be possible to find the day at which this specific issue was mailed to the subscribers. At that time, there was no simultaneous publication in a digital form. In the end of the nineties, the articles were with some delay stored electronically in databases and delivered to customers around the world through third parties, but the date of publication should be counted as the day of the paper version.

I hope these informations might be helpful.

Yours sincerely

Lars Alldén

Rights Director Aschehoug Agency

representing Aschehoug, Oktober and Universitetsforlaget

P.O.Box 363 Sentrum

NO-0102 Oslo, Norway

Direct phone: + 47 22 400 321

e-mail: lars.allden@aschehougagency.no

From: Jean Lee [mailto:jeanl@speckmanlaw.com]

Sent: Wednesday, October 20, 2004 10:26 PM

To: Agency

Cc: Ann Speckman

Subject: 1997 Universitetsforlaget publication

To Whom It May Concern:

I have been trying to find some information regarding *Cephalalgia*, a journal that is currently published by Blackwell Publishing Ltd. but was previously published by Universitetsforlaget/Scandinavian University Press. Specifically, I am trying to find the earliest date that the December 1997 issue (Volume 7, Number 8) became publicly available anywhere in the world, in print or electronic form. Date-stamped photocopies of this issue sent to us by two American university libraries indicate that the issue was received on January 6, 1998. I would, however, like to confirm with the publisher the actual distribution date and/or publication date, if the distribution date is unavailable.

I realize that, because Universitetsforlaget is no longer the publisher of *Cephalalgia*, production and distribution information from 1997 may no longer be available. Nevertheless, any assistance on this matter would be greatly appreciated. Thank you.

Best Regards,

Jean Lee, General Assistant

11/23/2004

Speckman Law Group PLLC
1501 Western Ave., Suite 100
Seattle, WA 98101
Tel. 206.382.1191
Fax. 206.382.2669
jeanl@speckmanlaw.com

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EXHIBIT B3

Date-stamped cover page from University of Oslo Library



UNIVERSITY OF OSLO LIBRARY

TELEFAX

To: Speckman Law group ATTN: Jean Lee

Fax no.: 01.206.382.2669

From: Library of Medicine and Health

No. of pages (incl.this): 9

Date: 25.10.2004

Library of Medicine and Health

P.O.Box 1115 Blindern
NO-0317 Oslo, Norway

Telephone: +4723074420

Telefax: +4723074430

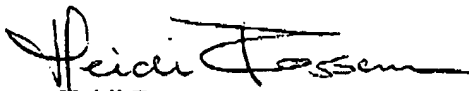
Email: umh@ub.uio.no

URL: <http://www.ub.uio.no/umh/>

Message:

Article from Cephalagia 1997;17(8):826-32

Yours sincerely


Heidi Fossum

RECEIVED

OCT 25 2004

SPECKMANLAWGROUP

UNIVERSITETET I OSLO

13 JAN 1998

HISTORISKE FAKULTETSDIOTEN

Editor-in-Chief:
KMA Welch, Detroit, USA

European Editors:
Dr L Edvinsson, Lund, Sweden
Dr M Wilkinson, London, UK

Assistant Editor:
Dr Nabih M. Ramadan
Cincinnati, OH, USA

Controversies Editor:
Dr Gretchen E. Tietjen
Toledo, OH, USA

Historical Section Editor:
Dr H. Isler, Zürich, Switzerland

Editorial Manager:
H. Ryan, Detroit, USA

Cephalalgia

An International Journal of Headache

Cephalalgia is one of the leading journals in its field and provides rapid publication of high-quality articles on every aspect of headache. Original research papers are carefully selected for an international forum.

Contributions appearing in *Cephalalgia* are abstracted and indexed in, among others, Current Contents, Index Medicus and Excerpta Medica.

Recent articles

Work-related disability: results from the American migraine study.
WF Stewart, RB Lipton, D Simon

Familial hemiplegic migraine: a clinical comparison of families linked and unlinked to chromosome 19. GM Terwindt, RA Ophoff, J Haan, RR Frantz, MD Ferrari

Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries
I Jansen-Olesen, A Mortensen, L Edvinsson

Migraine without aura and migraine with aura are distinct clinical entities: a study of 484 male and female migraineurs from the general population
MB Russell, BK Rasmussen, K Feinger, J Olesen

Visit *Cephalalgia*'s home page on the World Wide Web at <http://www.scup.no/cephalgia>

Join the International Headache Society

Cephalalgia is the official journal of the International Headache Society (IHS). Benefits of IHS membership include:

- Free subscription to *Cephalalgia* and its supplements
- IHS newsletter
- Information on IHS congresses, meetings, fellowships and other awards
- News of other international activities related to headache
- Early access to IHS guidelines and other publications on Classification, Clinical Trials, Treatment, Ethics and other important issues

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The Netherlands



SCANDINAVIAN
UNIVERSITY PRESS

All business communications to *Cephalalgia* and IHS should be sent to:
Scandinavian University Press, PO Box 2959 Toyen, N-0608 Oslo, Norway.
Tel: +47 22 57 54 00. Fax: +47 22 57 53 53. E-mail: subscription@scup.no
U.S. address: Scandinavian University Press North America, 875 Massachusetts Ave., Ste. 84, Cambridge, MA 02139, USA. Tel: 617 497 6515. Fax: 617 354 6875. Toll-free tel: 800 498 2877. E-mail: 75201.571@compuserve.com

Non-member subscription (Institutions) Application for membership (Individuals only)

- ☐ Please enter our subscription to *Cephalalgia* from Vol. 17, No. 1, 1997. Subscription rate for 1997: US\$ 300 (In Scandinavia, NOK 1700,-)
- ☐ Please send me a free sample copy of *Cephalalgia*.
- ☐ Please send me an application form for membership to the IHS, which also includes free subscription to *Cephalalgia*. (Membership is on a calendar-year basis. Combined membership/subscription fee for 1997: US\$ 115.)

Publication Information: ISSN 0333-1024. Eight issues per year. Free supplements. Postage and airspeeded delivery worldwide included. Prepayment is required.

Please check one box: ☐ Cheque enclosed ☐ Please send invoice

Please charge my: ☐ VISA ☐ Eurocard/Mastercard ☐ AmEx ☐ Diners Club

Card No.: Expiry Date:
(Please make the cheque payable to Scandinavian University Press and staple it to your order form.)

Name/ Address (BLOCK LETTERS):

Signature: Fax:

7-220 x

EXHIBIT B4

BIBSYS database receipt records



Record 822122316

- BibSøk Nett -

[Simple search](#)
[Advanced srch](#)
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[Instructions](#)

Cephalalgia (trykt utg.)

Holdings for all BIBSYS Libraries:

Owner	Position	Status	Dokid
1 UBIT - GUNNERUS	T *61(05) Cep	(Også tilgjengelig online for NTNU) [Korttid] 1 (1981)-15(1995)	82b012231
2 UBIT - MED	Tidsskr Cel	[Ikke utlån] 1(1981)-19(1999)nr 10.	94f000228
3 UBTØ - RMH	tids Cep	[Ikke utlån] 1(1981)nr 2-4,2(1982)1-4,3(1983)nr 1,3,5(1985)suppl.3,13(1993)-14(1994)	93c019203
4 UBB - UBBMED	ZG Cep	(Oppstilt i kompaktmag.) 1(1981)-19(1999)	88d007647
5 NBO - NBO	NAZ 650/5	(Til bruk på NB Oslos lesesal) / RECEIVED AT THE LIBRARY 1(1981)-19(1999)nr 10	95ga01902
6 UMH - UMH		(Oppstilt alfabetisk) [Ikke utlån] 1(1981)-20(2000)	96za00428
7 NBR - NBR/PL	1539	(Sikringseksemplar.) [Ikke utlån] 10(1990)nr 1-19 (1999)nr 10	93sa13197
8 NBR - NBR/GB		Gjenbrukseksemplar - 10(1990)nr 1-13(1993)	98sc02479
9 NBR - NBR/DEP		N [Ikke utlån] 10(1990)nr 2- .	90sd01230
10 SØRSYK/A - SØRSYK/A		(Ukpl.) - 16(1996)-23(2003)	04zg00245



Record 94f000228

- BibSøk Nett -

[Simple search](#) [Advanced srch](#) [Journals](#) [Instructions](#)
Cephalalgia (trykt utg.)**Registered issues:**

Year	Vol.	No.	Received	Expected	Partstatus/date	Remark
1999	19	10	2000-02-25			
1999	19	9	1999-12-29			
1999	19	8	1999-12-03			
1999	19	7	1999-10-22			
1999	19	6	1999-08-16			
1999	19	5	1999-07-26			
1999	19	4	1999-07-12			
1999	19	3	1999-05-25			
1999	19	2	1999-04-26			
1999	19	1	1999-04-14			
1998	18	10	1999-02-04			
1998	18	9	1999-01-07			
1998	18	8	1998-11-17			
1998	18	7	1998-10-16			
1998	18	6	1998-09-04			
1998	18	5	1998-07-23			
1998	18	4	1998-06-26			
1998	18	3	1998-05-13			
1998	18	2	1998-04-21			
1998	18	1	1998-02-23			
1997	17	8	1998-01-09			BEM1
1997	17	7	1997-11-24			
1997	17	6	1997-10-24			
1997	17	5	1997-08-07			
1997	17	4	1997-06-20			
1997	17	3	1997-05-28			
1997	17	2	1997-04-29			
1997	17	1	1997-02-27			
1996	16	8	1997-02-07			BEM2
1996	16	7	1996-12-09			
1996	16	6	1996-11-25			
1996	16	5	1996-08-22			

1996 16	4		1996-06-24
1996 16	3		1996-05-21
1996 16	2	April	1996-05-13
1996 16	1	Februar	1996-05-13
1995 15	6	Desember	1995-12-14
1995 15	5	Oktober	1995-11-02
1995 15	4	August	1995-11-02
1995 15	3	Juni	1995-11-02
1995 15	2	April	1995-08-24
1995 15	1	Februar	1995-03-08
1994 14	6	Desember	1994-12-28
1994 14	5	Oktober	1994-11-07
1994 14	4	August	1994-08-26
1994 14	3	Juni	1994-08-26
1994 14	2	April	1994-08-26
1994 14	1	Februar	1994-08-26
1993 13	6	Desember	1994-08-26
1993 13	5	Oktober	1994-08-26
1993 13	4	August	1994-08-26
1993 13	3	Juni	1994-08-26
1993 13	2	April	1994-08-26
1993 13	1	Februar	1994-08-26

Private remarks (for the Library):**BEM1 (1997:17:8) : Index****BEM2 (1996:16:8) : Index**



Record 88d007647

- BibSøk Nett -

[Simple search](#)
[Advanced srch](#)
[Journals](#)
[Instructions](#)

Cephalalgia (trykt utg.)

Registered issues:

Year	Vol.	No.	Received	Expected	Partstatus/date	Remark
1999	19	10	2000-02-28			
1999	19	9	1999-12-28			
1999	19	8	1999-12-08			
1999	19	7	1999-10-25			
1999	19	6	1999-08-16			
1999	19	5	1999-07-27			
1999	19	4	1999-07-15			
1999	19	3	1999-05-25			
1999	19	2	1999-04-27			
1999	19	1	1999-04-13			
1998	18	10	1999-02-16			
1998	18	9	1999-01-12			
1998	18	8	1998-12-01			
1998	18	7	1999-02-23			
1998	18	6	1998-09-15			
1998	18	5	1998-08-17			
1998	18	4	1998-07-09			
1998	18	3	1998-05-26			
1998	18	2	1998-04-22			
1998	18	1		1998-04-24	NBR	
1997	17	8	1998-02-24			
1997	17	7	1997-12-01			
1997	17	6	1997-11-26			
1997	17	5	1997-09-23			
1997	17	4	1997-07-21			
1997	17	3	1997-07-02			
1997	17	2	1997-06-02			
1997	17	1	1997-04-29			
1996	16	8	1997-02-11			
1996	16	7	1996-12-10			
1996	16	6	1996-11-25			
1996	16	5	1996-09-17			

1996 16	4		1996-07-08
1996 16	3		1996-06-18
1996 16	2	April	1996-05-03
1996 16	1	Februar	1996-02-20
1995 15	6	Desember	1996-01-08
1995 15	5	Oktober	1995-11-01
1995 15	4	August	1995-09-04
1995 15	3	Juni	1995-08-22
1995 15	2	April	1995-05-30
1995 15	1	Februar	1995-05-30



Record 96za00428

- BibSøk Nett -

[Simple search](#) [Advanced srch](#) [Journals](#) [Instructions](#)**Cephalalgia (trykt utg.)****Registered issues:**

Year	Vol.	No.	Received	Expected	Partstatus/date	Remark
2001	20	10	2001-04-18			
2000	20	9	2001-02-15			
2000	20	8	2001-01-25			
2000	20	7	2000-12-01			
2000	20	6	2000-10-26			
2000	20	5	2000-10-10			
2000	20	4	2000-09-08			
2000	20	3	2000-09-08			
2000	20	2	2000-08-17			
2000	20	1	2000-04-28			
1999	19	10	2000-02-04			
1999	19	9	1999-12-10			
1999	19	8	1999-11-16			
1999	19	7	1999-10-05			
1999	19	6	1999-08-19			
1999	19	5	1999-07-07			
1999	19	4	1999-06-17			
1999	19	3	1999-05-21			
1999	19	2	1999-04-14			
1999	19	1	1999-03-23			
1998	18	10	1999-02-01			
1998	18	9	1999-01-04			
1998	18	8	1998-11-16			
1998	18	7	1998-10-09			
1998	18	6	1998-09-03			
1998	18	5	1998-07-21			
1998	18	4	1998-06-23			
1998	18	3	1998-05-12			
1998	18	2	1998-03-19			
1998	18	1	1998-02-24			
1997	17	8	1998-01-08			
1997	17	7	1997-11-19			

1997 17	6	1997-10-22
1997 17	5	1997-07-29
1997 17	4	1997-06-19
1997 17	3	1997-05-26
1997 17	2	1997-04-23
1997 17	1	1997-02-21
1996 16	8	1997-01-03
1996 16	7	1996-11-21
1996 16	6	1996-10-21
1996 16	5	1996-08-22
1996 16	4	1996-06-20
1996 16	3	1996-05-15



Record 93sa13197

- BibSøk Nett -

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Cephalalgia (trykt utg.)**Registered issues:**

Year	Vol. No.	Received	Expected	Partstatus/date	Remark
1999			EMB		BEM1
1999	19 10	2000-02-14			
1999	19 9	1999-12-15			
1999	19 8	1999-11-24			
1999	19 7	1999-10-11			
1999	19 6	1999-08-10			
1999	19 5	1999-07-16			
1999	19 4	1999-06-23			
1999	19 3	1999-05-11			
1999	19 2	1999-04-19			
1999	19 1	1999-03-24			
1998	18 10	1999-02-08	EMB		BEM2
1998	18 9	1999-01-06			
1998	18 8	1998-11-17			
1998	18 7	1998-10-14			
1998	18 6	1998-09-04			
1998	18 5	1998-08-06			
1998	18 4	1998-07-01			
1998	18 3	1998-05-18			
1998	18 2	1998-04-08			
1998	18 1	1998-03-16			
1997	17 8	1998-02-04			
1997	17 7	1997-11-25			
1997	17 6	1997-11-18			
1997	17 5	1997-09-15			
1997	17 4	1997-07-11			
1997	17 3	1997-06-23			
1997	17 2	1997-05-13			
1997	17 1	1997-03-18			
1996	16 8	1997-02-03			
1996	16 7	1996-12-04			
1996	16 6	1996-11-13			

1996 16	5	1996-09-09
1996 16	4	1996-06-27
1996 16	3	1996-06-05
1996 16	2	1996-04-17
1996 16	1	1996-02-13
1995 15	6	1995-12-22
1995 15	5	1995-10-20
1995 15	4	1995-08-25
1995 15	3	1995-08-10
1995 15	2	1995-05-23
1995 15	1	1995-03-21
1994 14	6	1995-01-11
1994 14	5	1995-01-09
1994 14	4	1995-01-09
1994 14	3	1995-01-09
1994 14	2	1995-01-09
1994 14	1	1995-01-09
1993 13	6	1995-01-09
1993 13	5	1995-01-09
1993 13	4	1995-01-09
1993 13	3	1995-01-09
1993 13	2	1995-01-09
1993 13	1	1995-01-09
1992 12	6	1995-01-09
1992 12	5	1995-01-09
1992 12	4	1995-01-09
1992 12	3	1995-01-09
1992 12	2	1995-01-09
1992 12	1	1995-01-09
1991 11	6	1995-01-09
1991 11	5	1995-01-09
1991 11	4	1995-01-09
1991 11	3	1995-01-09
1991 11	2	1995-01-09
1991 11	1	1995-01-09
1990		
1990 10	6	1995-01-09
1990 10	5	1995-01-09
1990 10	4	1995-01-09
1990 10	3	1995-01-09
1990 10	2	1995-01-09

EMB

BEM3

1990 10 1 1995-01-09

Private remarks (for the Library):

BEM1 (1999) : B314-68-4-5

BEM2 (1998:18:10) : B314-68-4-5

BEM3 (1990) : B314-68-4-5

EXHIBIT B5

E-mail correspondence with Marianne Siener

Jean Lee

From: Brophy, Marianne [Marianne.Brophy@umassmed.edu]
Sent: Thursday, November 18, 2004 7:48 AM
To: jeanl@speckmanlaw.com
Subject: Receipt date question on Cephalalgia

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Ms. Lee,

Our check-in date on Cephalalgia v.17 no.8 Dec 1997 is Jan. 5 1998. Our policy is to put the journal out into the public area of the library on the same day it is checked into our system.

I hope this information helps.

Thank You,

Marianne Siener
Library - Serials
Univ of Mass Med School
55 Lake Ave No
Worcester, MA 01655
(508) 856-2388

Patron's Email: jeanl@speckmanlaw.com

Patron's Phone Number: (206) 382-1191

Question: I am trying to determine the earliest date that the December 1997 issue of the journal Cephalalgia (Volume 17, Number 8), ISSN 0333-1024, became publicly available anywhere in the world, in either print or electronic format. As Cephalalgia, at the time of this issue, was published in Oslo, Stockholm, Copenhagen, Oxford, and Boston, we are hoping to find accessions dates for national, academic, or research libraries located in the cities of publication, under the presumption that these would be among the first subscribers to receive the issue.

Is there any means of determining receipt dates for this issue (e.g. accessions or receipt records, date-stamping of journal cover)? If so, any assistance you could provide in confirming library receipt date(s) of this issue would be greatly appreciated. Thank you.

Best Regards,

Jean Lee, General Assistant
Speckman Law Group PLLC

EXHIBIT B6

E-mail correspondence with Hélène Gautier Gentès

EXHIBIT B7

E-mail correspondences with Liselotte Jørgensen and Per Morten Sørensen

Jean Lee

From: Liselotte Jørgensen [lij@DNLB.DK]
Sent: Tuesday, November 16, 2004 11:23 PM
To: Jean Lee
Subject: SV: question about library receival date for serial (Cephalalgia)
Follow Up Flag: Follow up
Flag Status: Flagged

Dear Mrs Jean Lee

vol 17 no 8 was received jan.9, 1998 according to my records.

Yours sincerely

Liselotte Jørgensen
Serials Department
lij@dnlb.dk

Danish National Library
of Science and Medicine
49 Nørre Allé
DK 2200 Copenhagen N

Fra: Jean Lee [<mailto:jeanl@speckmanlaw.com>]
Sendt: 16. november 2004 21:35
Til: Henriette Fog; Per Morten Sørensen; doc@dnlb.dk; Udlaan: Postkasse; Liselotte Jørgensen; Susanne Lindow
Emne: question about library receival date for serial (Cephalalgia)

To Whom It May Concern:

We are trying to determine the earliest date that the December 1997 issue of the journal Cephalalgia (Volume 17, Number 8), became publicly available anywhere in the world, in either print or electronic format. As Cephalalgia, at the time of this issue, was published by Scandinavian University Press/Universitetsforlaget in Oslo, Stockholm, Copenhagen, Oxford, and Boston, we presume that the Danish National Library of Science and Medicine would be among the first subscribers to receive the print version of this issue.

Does the Library have any method of determining the date this particular issue was received (e.g. processing records, date-stamping of journal cover)? If so, any assistance you could provide in confirming the library's receival date of this issue would be greatly appreciated. Thank you.

Best regards,

Jean Lee, General Assistant
Speckman Law Group PLLC
1501 Western Ave., Suite 100
Seattle, WA 98101
Tel. 206.382.1191
Fax. 206.382.2669

11/23/2004

jeanl@speckmanlaw.com

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11/23/2004

Jean Lee

From: Per Morten Sørensen [PMS@DNLB.DK]
Sent: Monday, November 22, 2004 12:00 AM
To: Jean Lee
Subject: SV: question about library receival date for serial (Cephalalgia)
Follow Up Flag: Follow up
Flag Status: Flagged

Received Jan-09-1998.

Best regards,

Per Morten Sørensen

Fra: Jean Lee [mailto:jeanl@speckmanlaw.com]

Sendt: 16. november 2004 21:35

Til: Henriette Fog; Per Morten Sørensen; doc@dnlb.dk; Udlaan: Postkasse; Liselotte Jørgensen; Susanne Lindow

Emne: question about library receival date for serial (Cephalalgia)

To Whom It May Concern:

We are trying to determine the earliest date that the December 1997 issue of the journal Cephalalgia (Volume 17, Number 8), became publicly available anywhere in the world, in either print or electronic format. As Cephalalgia, at the time of this issue, was published by Scandinavian University Press/Universitetsforlaget in Oslo, Stockholm, Copenhagen, Oxford, and Boston, we presume that the Danish National Library of Science and Medicine would be among the first subscribers to receive the print version of this issue.

Does the Library have any method of determining the date this particular issue was received (e.g. processing records, date-stamping of journal cover)? If so, any assistance you could provide in confirming the library's receival date of this issue would be greatly appreciated. Thank you.

Best regards,

Jean Lee, General Assistant
Speckman Law Group PLLC
1501 Western Ave., Suite 100
Seattle, WA 98101
Tel. 206.382.1191
Fax. 206.382.2669
jeanl@speckmanlaw.com

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11/23/2004

EXHIBIT B8

E-mail correspondence with Reference Division of UCLA Biomedical Library

Jean Lee

From: Reference Division, UCLA Biomedical Library [bio_pc@library.ucla.edu]
Sent: Thursday, November 18, 2004 12:00 PM
To: jeanl@speckmanlaw.com
Subject: Re: Reference Question

Follow Up Flag: Follow up
Flag Status: Flagged

This issue is stamped with a received date of 1/9/1998.

--On Wednesday, November 17, 2004 5:46 PM -0800 jeanl@speckmanlaw.com wrote:

>
> NAME: Jean Lee
>
> TELEPHONE: 206-382-1191
>
> STATUS: other
>
> QUESTION: I am trying to determine the earliest date that the December
> 1997 issue of the journal Cephalalgia (Volume 17, Number 8), ISSN
> 0333-1024 became publicly available anywhere in the world, in either
> print or electronic format. As the former publisher is unable to
> provide an exact distribution date, I am hoping to find receival dates
> of this issue for various national, academic, and research libraries
> in order to obtain an estimate of the earliest date of public
> availability.
>
> Does the UCLA library currently have any method of determining the
> date this particular issue was received (e.g. receipt or accessions
> records, date-stamping of journal cover)? If so, any assistance you
> could provide in confirming the library's receival date of this issue
> would be greatly appreciated. Thank you.
>
>
> Best regards,
>
> Jean Lee, General Assistant
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> SUBMITTED ON: 17-Nov-04 05:46 PM
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EXHIBIT B9

E-mail correspondence with Manon Cockwell

Jean Lee

From: Cockwell, Manon [Manon.Cockwell@nrc-cnrc.gc.ca] on behalf of CISTI, CIRC [CISTI.CIRC@nrc-cnrc.gc.ca]
Sent: Thursday, November 18, 2004 11:04 AM
To: 'jeanl@speckmanlaw.com'
Subject: RE: question regarding accession date of serial Cephalalgia Dec. 1997
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I hope that the information provided to you will prove to be useful. If you should have any further questions, please do not hesitate to contact me and good luck in your search.

Sincerely yours/Amicalement vôtre,

Manon Cockwell

On-site Services Officer / Agente aux services sur place / CISTI/ICIST M-55

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-----Original Message-----

From: Jean Lee [mailto:jeanl@speckmanlaw.com]

Sent: November 18, 2004 12:21 PM

To: info.cisti@nrc.ca

Subject: question regarding accession date of serial Cephalalgia Dec. 1997

Hello,

We are trying to determine the earliest date that the December 1997 issue of the journal Cephalalgia

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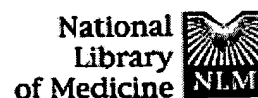
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Best regards,

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Cortical spreading depression and migraine: new insights from imaging?

James MF, Smith JM, Boniface SJ, Huang CL, Leslie RA.

Neuroscience Research, GlaxoSmithKline, New Frontiers Science Park (North), Third Avenue, Harlow, Essex, UK, CM19 5AW.

The possibility that spreading depression (SD) of cortical activity, a phenomenon observed in all vertebrates, causes the aura of migraine remains an open question in spite of nearly half a century of investigation. SD is also thought to be associated with the progressive neuronal injury observed during cerebral ischaemia. Thus, the ability to detect and investigate SD in humans might prove clinically significant. Animal studies of cortical spreading depression (CSD) have benefited greatly from the advent of relatively non-invasive imaging techniques. The use of these new imaging techniques for clinical studies will accelerate progress in this area of neurobiology.

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Nov 16 2004 07:00:47

Is There a Correlation Between Spreading Depression, Neurogenic Inflammation, and Nociception That Might Cause Migraine Headache?

Andrea Ebersberger,¹ Hans-Georg Schaible,¹ Beate Averbeck,² and Frank Richter¹

The time course of propagation of scotoma and blood flow changes during migraine aura parallels the phenomenon of cortical spreading depression (CSD). It was proposed that CSD generates a sterile neurogenic inflammation in the meninges, which may then lead to the activation or sensitization of nociceptors, thus generating headache. We performed rat experiments in which the effect of CSD on plasma extravasation in the dura mater and on neuronal activity in deep laminae of the trigeminal nucleus was assessed *in vivo*. CSD did not alter dural plasma extravasation measured by means of bovine serum albumin-coupled fluorescein ($n = 17$ rats) compared to the CSD-free contralateral side. In an *in vitro* model, the application of KCl to the dura at concentrations extracellularly found during CSD did not alter the release of calcitonin gene-related peptide and prostaglandin E_2 from the dura. In 33 rats, neither single CSDs nor a series of CSDs altered ongoing neuronal activity or mechanical and/or thermal sensitivity of the deeply located neurons to stimulation of their receptive fields in the dura mater. These results are at variance with data that showed increased c-Fos labeling in superficial laminae of the trigeminal nucleus following CSD. They do not suggest that CSD initiates migraine headache *via* neurogenic inflammation.

Ann Neurol 2001;49:7-13

In classical migraine, many patients experience an aura in the 30 to 45 minutes before the headache starts. The aura often consists of visual scotomata that propagate across the visual field and are often accompanied by somatosensory symptoms.¹ During the aura a transient oligemia is spreading across the cortex.^{1,2}

In 1944, Leão reported a neuronal phenomenon in the cortex of rabbits that he called cortical spreading depression (CSD) of electroencephalographic activity.³ This is a slow transient depolarization of neurons that spreads unilaterally across one hemisphere from a primary focus like a wave and is followed by a longer-lasting depression of neuronal activity. Because CSD propagates at a similar velocity as the scotomata and the transient cortical oligemia in humans, CSD was proposed to be the mechanism underlying the migraine aura.^{1,4}

The headache in migraine is thought to result from neuronal nociceptive activity in the trigeminovascular system, that is, the meninges.⁵ Moskowitz and Macfarlane proposed that CSD could depolarize primary meningeal afferents and thus cause a perivascular re-

lease of substance P and calcitonin gene-related peptide (CGRP) from the sensory endings.⁶ These neuropeptides cause a sterile neurogenic inflammation. In animal models neurogenic inflammation can be mimicked by applying inflammatory mediators to the meninges.⁷⁻¹⁰ In humans CGRP was found to be enhanced in the venous outflow from the head during the headache attack.¹¹ Antimigraine drugs, such as sumatriptan, attenuated the neuropeptide release in humans¹² and the experimentally induced plasma extravasation in animals.⁶

Because in humans the aura usually, but not always, precedes the migraine pain,¹³ the question arises whether the putative neuronal mechanism of the aura, the CSD, and the nociceptive activity in the trigeminal system are linked and, if so, how. Characteristically, during a CSD in a rat, extracellular potassium rises up to 40 to 60 mM in the gray matter of the cortex.¹⁴ It is interesting to note that a transient elevation of potassium levels can also be measured epidurally (up to 8 mM) and subdurally (up to 15 mM).¹⁵ Thus, elevated potassium levels may depolarize the primary afferent

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neurons in the meninges and lead to the perivascular release of neuropeptides. Neurogenic inflammation could then activate the nociceptive system and generate the pain.⁶

However, the link between CSD and nociceptive activity has never been directly shown in electrophysiological experiments. Therefore, in the anesthetized rat we recorded neuronal activity from secondary sensory neurons in the trigeminal nucleus caudalis with input from the meninges and tested whether nociceptive neurons at this site could be activated and/or sensitized as a consequence of CSD. Furthermore, we assessed whether CSD can cause an induction of plasma extravasation in the meninges and whether the potassium concentrations measured sub- and epidurally during CSD are sufficient to elicit the release of CGRP and prostaglandin E₂ (PGE₂) in the meninges.

Methods

Animal Preparation

The present study was approved by the animal protection committee and the regional government of Thuringen (registration no. 02-04/98). Male Wistar rats (300–450 gm) were anesthetized with intraperitoneal sodium thiopentone (100 mg/kg Trapanal, Byk Gulden, Konstanz, Germany). Supplemental intraperitoneal doses (20 mg/kg) were given to maintain areflexia. The trachea and the left femoral vein and artery were cannulated. The animals breathed spontaneously. Mean arterial blood pressure was continuously monitored. Body temperature was kept at 37°C using a feedback-controlled system. The head of the animal was fixed in a stereotaxic frame, and the apical and parietal parts of the skull were exposed following a median incision. Over the left hemisphere the dura mater was exposed from bregma to lambda (from midline spanning 5 mm laterally) using a minidrill under saline cooling (Fig 1) and kept moist with Tyrode's solution throughout the experiment. Two further openings (diameter 2 mm) were made at 2 mm anterior and lateral from bregma and at 3 mm anterior and 4 mm lateral from bregma on the left (Fig 1, sites 1 and 2) and as a sham control on the right hemisphere. Underneath these openings the dura mater was incised. The brainstem was exposed by standard surgical procedures.

Recording of Direct Current Potentials

An Ag/AgCl reference electrode containing 2 M KCl was placed on the nasal bone. Intracortical direct current (DC) potentials were recorded using glass micropipettes filled with 150 mM NaCl. Two or four pipettes were glued together, their tips staggered by 800 or 400 μ m, respectively. The electrode assembly was lowered through the frontomedial opening (Fig 1, site 2) into the gray matter to a depth of 1600 μ m. The signals were recorded using high-impedance amplifiers (pH/Ion Amplifier Model 2000, A-M Systems Inc, Carlsborg, WA) and stored on personal computer. Through the frontolateral opening (Fig 1, site 1) single CSDs were elicited by a pinprick into the cortex with a nee-

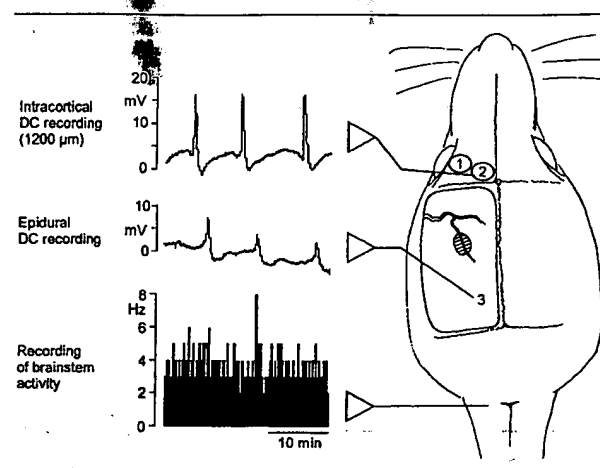


Fig 1. Diagram of the experimental setup to follow the propagation of cortical spreading depression (CSD) across one hemisphere of the rat cortex during recording of brainstem neuronal activity. The right panel shows a schematic view at the top of a rat skull that was opened at three sites (1–3) to expose the dura. At site 1, CSD was elicited. At site 2, direct current (DC) electrodes were inserted into the gray matter to record intracortical DC potentials. At site 3, the DC potential was recorded on the dura. The brainstem was exposed to record neuronal activity in the caudal trigeminal nucleus. Receptive fields of brainstem neurons were mapped on the dura. The hatched area gives a typical example. For this experiment, original recordings at sites 2 and 3 and in the brainstem are depicted in the left panel.

dle (diameter 0.5 mm). Repetitive CSDs were elicited by a drop of 5 μ l 2 M KCl onto the pia mater.

Brainstem Recordings

Either single or multiple neurons were recorded in the caudal trigeminal nucleus with carbon fiber microelectrodes. Amplified action potentials were recorded on-line with a personal computer (interface card DAB 1200, Microstar Laboratories Inc, Bellevue, WA) using the spike/spidi software package.¹⁶

Because dural and corneal afferents converge onto brainstem neurons,¹⁷ we touched the cornea to search for neurons. Neurons with corneal input were then tested for dural input by touching the dura with a blunt glass rod. To avoid desensitization of the primary afferents, the number of mechanical stimuli was kept at a minimum. In an identified neuron, spontaneous activity was recorded for 10 minutes. The facial receptive field was tested with mechanical stimuli. In neurons without spontaneous activity, the mechanical threshold on the dura was assessed with von Frey hairs. With a feedback-controlled stimulator (Department of Physiology and Experimental Pathophysiology, University of Erlangen), four ramp-shaped heat stimuli (continuous increase from 32°C to 52°C in 25 seconds) were applied to the dura mater to determine the thermal threshold of the neuron. After the characterization of a neuron, three single CSDs were elicited at intervals of 20 minutes, followed by application of KCl (see above) to the cortex while the neuron was continuously monitored. KCl application was repeated when repetitive

CSD activity stopped. Two hours after the initial KCl application, the characterization of the neuron under study was repeated.

Plasma Extravasation

Thirty minutes before the first CSD was elicited, 50 mg/kg bovine serum albumin labeled with fluorescein isothiocyanate (BSA-FITC, Sigma, Steinheim, Germany) diluted in 1 ml phosphate-buffered saline solution (pH 7.4, 0.1 M) was applied intravenously. In 7 animals, CSDs were then elicited by pinpricks at intervals of 20 minutes for 3 hours. In 10 animals we evoked CSDs with prick and KCl (discussed earlier). As a control, 6 animals were prepared as described except for the insertion of DC electrodes, and, 30 minutes after infusion of BSA-FITC, 5 μ l of substance P 10^{-5} M was applied to the intact and exposed dura mater for 5 minutes. This procedure was repeated three times at intervals of 20 minutes. After the experiment, rats were intravenously perfused with phosphate-buffered saline solution (37°C) at 120 mm Hg. The dura mater was bilaterally removed (exposure of the dura is described earlier), dried, weighed, and incubated overnight in 2 ml isotonic saline solution (pH 11) under constant stirring.¹⁸ The fluorescence of this solution was measured by quantitative fluorimetry (Perkin Elmer LS-30, Perkin Elmer Corp, Norwalk, CT; excitation wavelength 490 nm; emission wavelength 520 nm). For each animal, results were expressed in nanograms of extravasated BSA-FITC per milligram of dried dura mater. Within each group, mean values of stimulated and nonstimulated sides were compared by a paired Student's *t* test.

Calcitonin Gene-Related Peptide and Prostaglandin E_2 Release

The release of CGRP and prostaglandin E_2 (PGE₂) was measured in an in vitro model previously described.⁹ Rats were decapitated, the skull was longitudinally divided into two halves, and the brain was removed. After washing and adapting to 37°C, the cavities were filled six times for 5 minutes with synthetic interstitial fluid. The third filling contained KCl at the following concentrations: 8.3 mM, 15 mM, 30 mM, or 40 mM KCl (pH 7.2). In one experimental group, 15 mM KCl was applied at pH 6.8. CGRP and PGE₂ concentrations in the five eluates were determined by enzyme immunoassay (EIA) (CGRP-EIA from SPIBio Company, Paris, France, minimum detection level 2 pg/ml; PGE₂-EIA from Brune et al,¹⁹ minimum detection level 10 pg/ml). For statistical analysis within groups, the means of the second samples (prestimulation control) and third samples (stimulated preparation with maximal release) were compared using the Wilcoxon matched pairs signed rank test.

Results

Recording of Cortical Spreading Depression

A pinprick applied to the triggering site (Fig 1, site 1) evoked CSD-related DC shifts in the adjacent and remote cortex (Fig 1, site 2). Such DC potentials typically reached maxima of about 15 to 25 mV.²⁰ In five experiments, DC potentials of 6 to 8 mV were additionally recorded at the dural surface with a blunt elec-

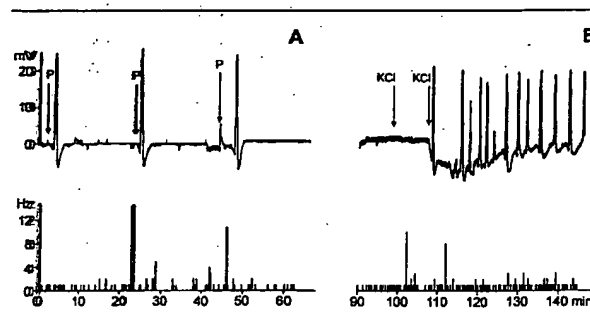
trode (Fig 1, site 3). The propagation velocity of CSDs was about 3 mm/minute (passing one hemisphere in less than 10 minutes).

Cortical Spreading Depression and Recordings from the Trigeminal Nucleus Caudalis

In 21 animals the dura was exposed, and single neurons in the brainstem with mechanosensory input from the dura were identified. Most of the neurons were located in Vc/Vi, with a few exceptions in Vc/C1. All the neurons were recorded in deeper laminae. Typically they had small mechanical receptive fields (diameter 1–3 mm) close to dural vessels (Fig 1, hatched area). Extracranial receptive fields were located at the cornea and the skin around the eye. This subset of neurons had already been described in detail elsewhere.²¹ When CSD was elicited by a prick into the cortex (Fig 2A, P), 8 of 11 neurons responded to the mechanical stimulus with a brief burst of action potentials due to the injury or the stretch of surrounding dural tissue (Fig 2A, lower trace). None of the neurons exhibited any activation correlated with the peak of CSD or in the 10-minute period following the maximum of the peak compared with the activity before the pinprick (mean values \pm SEM 0.53 ± 0.36 Hz versus 0.68 ± 0.49 Hz, respectively).

The discharges of the neurons also remained largely unaltered when repetitive CSDs were elicited over a period of 2 hours by applications of KCl to the cortical surface. Figure 2B shows a typical example. Since CSDs following KCl application occur at irregular intervals the mean number of CSDs (22.5 ± 8.2 per observation period) and the mean neuronal activity in the whole sample of neurons were averaged for intervals of 15 minutes. Figure 3A depicts the data from all neurons. The failure of CSD to cause CSD-related dis-

Fig 2. Specimen of a neuron that is not activated by the propagation of cortical spreading depression (CSD) across its receptive field. The upper panels show single CSDs (A) elicited by needle prick (P) and repetitive CSDs (B) elicited by topical application of KCl. The lower panels show the activity of the neuron. Immediate increases in discharge are seen following mechanical pricks or KCl application but not in relation to peaks in DC potentials.



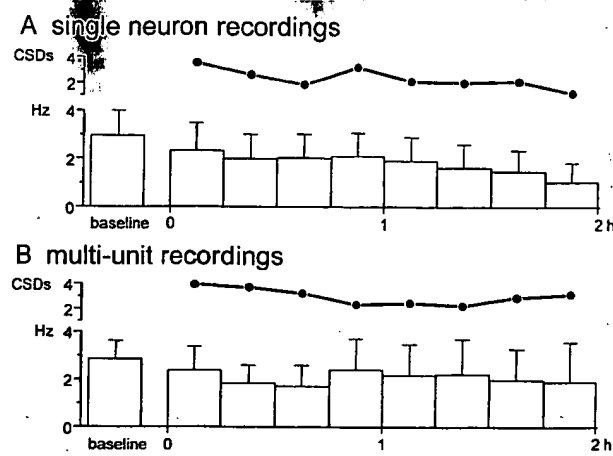


Fig 3. Neuronal activity was not altered by a series of cortical spreading depressions (CSDs). The columns show the mean neuronal activity \pm SEM during 15-minute intervals in 21 trigeminal single-cell recordings (A) and in 12 trigeminal multiunit recordings (B) at the beginning (baseline) and during the course of experiments in which repetitive CSDs were elicited by KCl. The line graphs in A and B show the mean number of CSD events during intervals of 15 minutes.

charges was also seen in multiunit recordings ($n = 12$) in which the temporal skull was left intact (Fig 3B). For the multiunit recordings, we searched for areas in the brainstem with intense neuronal input from the cornea because of the high convergence (80%) of corneal and dural afferents onto the same brainstem neurons.¹⁷

CSD may change the sensitivity of the neurons for stimuli rather than cause evoked discharges itself. The heat thresholds of single neurons were investigated in 18 animals before and after a series of mechanically and chemically evoked CSDs. Of 126 neurons with mechanoreceptive fields at the dura, only 19 (15%) responded to heat. Following a 3-hour series of repeated CSDs, heat thresholds in these neurons were elevated, from $37.9 \pm 3.7^\circ\text{C}$ to $42.2 \pm 4.7^\circ\text{C}$ (Fig 4A). Mechanical von Frey thresholds of neurons tested before and after the protocol of CSDs were either unchanged (8 neurons) or enhanced (5 neurons) (Fig 4B). Overall, the mean (\pm SEM) of the von Frey thresholds increased insignificantly from 1.75 ± 1.03 gm before CSDs to 2.05 ± 1.01 gm after CSDs ($n = 13$). Spontaneous activity remained constant throughout the experiment in 9 neurons, decreased in 7 neurons, and was enhanced in 3 neurons (Fig 4C). Mean values (\pm SEM) of spontaneous discharges at the beginning of the experiment did not differ from those after CSDs (3.08 ± 1.33 Hz versus 3.05 ± 1.34 Hz). Overall, the neurons were not sensitized but, rather, displayed a tendency to desensitize.

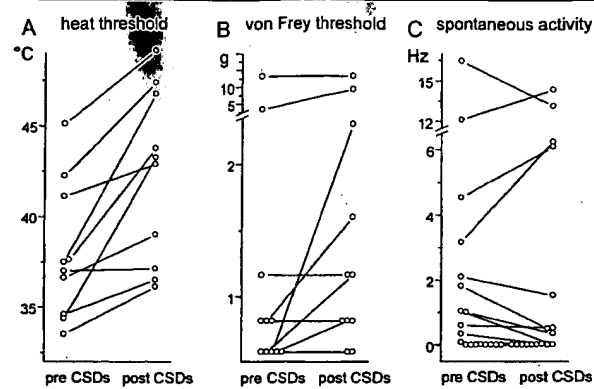
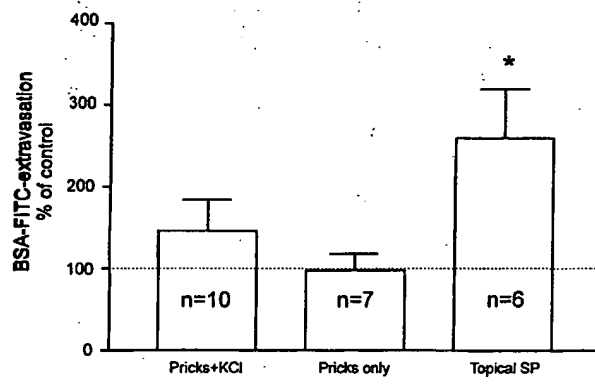


Fig 4. Neuronal sensitivity is not increased by a series of cortical spreading depressions (CSDs). Heat thresholds, mechanical von Frey thresholds, and spontaneous activity of brainstem trigeminal neurons are compared before and after a series of CSDs. Solid lines connect values of one neuron. (A) Heat thresholds significantly increased following CSDs ($p < 0.05$, Wilcoxon's matched paired signed rank test). (B) In most neurons, CSDs did not alter von Frey thresholds on the dural surface. Some of the neurons demonstrated enhanced mechanical thresholds. (C) No overall effect of CSDs on spontaneous neuronal activity was observed.

Dural Plasma Extravasation after Cortical Spreading Depression

The influence of a series of CSDs on dural plasma extravasation (Fig 5), a typical sign of neurogenic inflammation, was examined by measuring the extravasation of BSA-FITC. Fluorescein concentrations were compared in stimulated versus unstimulated sides of the brain. When substance P, a major stimulant of plasma

Fig 5. Plasma extravasation of bovine serum albumin labeled with fluorescein isothiocyanate (BSA-FITC) is not enhanced in dural tissue following CSDs. (Bars from left to right) Extravasation is given as percentage (\pm SEM) of the contralateral side after elicitation of CSDs by prick and KCl, after triggering of CSDs by pricks only, and after topical application of substance P (positive control). * indicates a significant difference between the stimulated and the nonstimulated sides ($p < 0.05$, Wilcoxon's matched paired signed rank test).

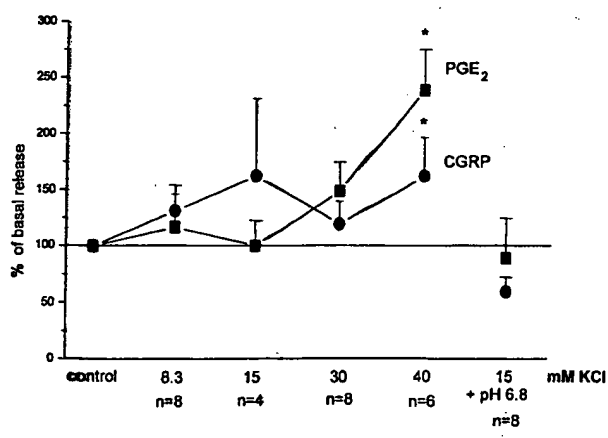


extravasation, was applied to the dura mater (positive control), BSA-FITC extravasation was significantly increased (8.7 ± 3.1 ng/mg versus 4.2 ± 1.5 ng/mg). When CSDs were mechanically elicited by a prick every 20 minutes for 3 hours, no difference in extravasation (9.1 ± 1.0 ng/mg versus 11.1 ± 2.6 ng/mg) was measured. A nonsignificant enhancement in BSA-FITC extravasation (11.0 ± 2.0 ng/mg versus 8.9 ± 1.5 ng/mg) was observed when CSDs were mechanically elicited three times, followed by 2 hours of chemically elicited CSDs.

Release of Calcitonin Gene-Related Peptide and Prostaglandin E₂ after KCl Administration

During CSD, the extracellular potassium concentration rises to 40 to 60 mM in the cortex.^{14,20} In addition, potassium concentration is elevated by 4.7 ± 3.3 mM epidurally and 15.4 ± 7.4 mM epicortically.¹⁵ Corresponding concentrations of KCl were used to stimulate the release of PGE₂ and CGRP in the dura. Only the stimulation with 40 mM KCl evoked a significant enhancement in PGE₂ and CGRP release (Fig 6). Even when the pH of a 15-mM KCl solution was lowered to 6.8 (because acidification possibly occurs during inflammatory conditions or reduced blood supply following CSD), PGE₂ and CGRP were not released from the dura mater.

Fig 6. KCl concentrations measured epidurally and epicortically during CSD do not stimulate release of prostaglandin E₂ (PGE₂) and calcitonin gene-related peptide (CGRP). Release of CGRP and PGE₂ (percentage of basal release before stimulation \pm SEM) into the fluid-filled isolated skull cavities after chemical stimulation with KCl solution (8.3 mM, 15 mM, 30 mM, and 40 mM at pH 7.2 and 15 mM at pH 6.8). The acidification of one 15-mM solution resembles conditions possibly occurring during inflammation of the dura. * indicates a significant increase in release compared to values before application of KCl ($p < 0.05$, Wilcoxon's matched paired signed rank test).



Discussion

We show that CSD of the cortex is not sufficient to trigger activation or sensitization of deeply located sensory second-order neurons in the trigeminal nucleus caudalis with input from the dura. Nor is CSD able to cause plasma extravasation in the meninges. Finally, elevation of potassium levels into the range observed epicortically and epidurally after cortical CSD does not enhance release of CGRP and PGE₂ from the dura. Collectively, these results do not support the general hypothesis that CSD could activate the trigeminovascular system by leading to neurogenic inflammation that ultimately causes migraine headache.²²

All of the brainstem neurons recorded in deep laminae responded immediately and briefly to mechanical stimulation of the dura mater, and 12 of 21 neurons were directly activated by the chemical stimulus when KCl was applied to the dura. Thus, the neurons were excitable before CSDs were triggered. However, no activation was seen that could be related to the process of CSD. Neurons were activated neither by few mechanically evoked CSDs (see also the recent findings of Lambert et al²³ in the cat) nor by multiple CSDs. Even when on average as many as 22.5 ± 8.2 CSDs crossed the receptive field of the brainstem neurons, ongoing activity of these neurons was not altered. The direct activation of dural afferents by the stimulus could also have evoked brainstem activation in another study that did not record DC potentials.²⁴ In accordance with this observation, Ingvar et al²⁵ suggested that the enhanced expression of the activity marker c-Fos in the brainstem after CSD²⁶ may be due to direct activation of dural afferents (by the injection needle and KCl) rather than to the mechanisms of CSD. The application of inflammatory mediators to the dural surface leads to an increase in mechanical and thermal sensitivity of the trigeminal neurons.^{7,27} By contrast, CSD did not increase the sensitivity of the neurons. Rather, heat thresholds were significantly elevated and mechanical thresholds tended to be increased after CSD. These elevations may be an expression of a desensitization of the primary afferents by either too low or too slowly increasing concentrations of potassium in the tissue.²⁸ In addition, spontaneous activity of the neurons did not increase, regardless of the experimental situation (i.e., single or multiunit recordings or exposure of the dura mater or not). Thus, CSD did not trigger nociception in the deeply located neurons in the trigeminal nucleus caudalis, which we investigated in this study.

The possibility that we may have missed neurons that are influenced by CSD must be considered. Indeed, the recorded neurons were located mainly in deeper laminae of the trigeminal nucleus caudalis, whereas the expression of c-Fos was found mainly in superficial laminae.²⁶ For several reasons, we believe that we have studied a population of neurons that is

important for trigeminal nociception. First, c-Fos is also expressed in deeper laminae. Second, in a previous study of the release of substance P in the trigeminal nucleus caudalis, release was maximal in the superficial laminae after chemical stimulation of the nasal mucosa, whereas release was maximal in the deeper laminae after chemical stimulation of the dura.²⁹ Third, we selected a population of neurons that had identified receptive fields in the dura. Still, the possibility remains that neurons preferentially activated in studies using c-Fos labeling (neurons in superficial laminae) exhibit different responses to CSD: they may be activated by CSD.⁶ However, it should be noted that CSD did not evoke plasma extravasation, and potassium levels seen during CSD did not cause release of CGRP and PGE₂. The latter data are in accord with the observation of unchanged CGRP levels in the venous outflow from the skull during the aura phase in migraineurs after experimental induction of an attack.³⁰ Thus, at least for deeply located neurons in the trigeminal nucleus, the sequence CSD followed by neurogenic inflammation followed by nociception is unlikely.

A final question concerns the relevance of the present findings to human migraine. Although some studies have provided evidence, based on blood flow measurements and functional MRI studies,³¹ that CSD exists in humans, others have failed to reveal changes before the onset of headache.^{32,33} It has also been shown that classical antimigraine drugs, such as sumatriptan³⁴ or vasodilator agents,³⁵ failed to affect CSD initiation or propagation in the cat. These findings also raise the question of whether CSD is important for the initiation of nociception.

This work was supported by Verbund für Klinische Forschung of the University of Jena (01 ZZ 9602, TP1.4).

We thank Mrs Helga Müller for excellent technical assistance.

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Cortical Spreading Depression: Its Role in Migraine Pathogenesis and Possible Therapeutic Intervention Strategies

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Cortical spreading depression (CSD) is a well-characterized phenomenon in experimental animals. Recent data show that CSD actually can occur in the injured human brain and compelling evidence is accumulating to support the concept that CSD is responsible for migraine aura. The aim of this review is to highlight recent key advances regarding our understanding of CSD in animal and human studies and its relevance to the pathophysiology of migraine and its potential treatment options.

Introduction

Cortical spreading depression (CSD) is a well-known experimental phenomenon that has been well characterized in animal models since it was first reported 50 years ago [1]. Spreading depression was first characterized in anesthetized rabbits as a slowly spreading suppression of electroencephalographic activity that was associated with marked pial vasodilatation [2]. Since these initial observations, CSD has been detected in a variety of animals including primates [3]; however, until recently, there was marked debate in the literature regarding whether it occurs in humans.

Spreading depression can be initiated by a traumatic stimulus applied to the cortical surface such as a blunt trauma or exposure to high $[K^+]_e$ excitatory amino acids or other factors such as endothelin. It is associated with an initial excitation of neuronal tissue followed by a refractory period. Marked changes occur in the extracellular ionic milieu with increases in K^+_e and H^+_e and decreases in $[Ca^{++}]_e$ [4], with a negative shift in extracellular dc potential of approximately 20 mV. Therefore, CSD could be described as a process of depolarization and repolarization with initially high $[K^+]_e$ producing disinhibition of voltage gated Mg^{++} ion blockade of the N-methyl-D-aspartate

(NMDA) channel. Following this activation, there is a period of repolarization when the neuron is refractory to further stimulation. This dynamic process of depolarization and repolarization allows progression of the wave of activity. In initial studies, it was demonstrated that CSD propagates at a rate of approximately 3 mm min^{-1} ($2-4 \text{ mm min}^{-1}$) across the cortical surface, providing a key feature of this phenomenon. A schematic representation of events involved in spreading depression is shown in Figure 1 [5]. CSD also gives rise to a characteristic pial vasodilatation [2] and a slowly spreading decrease in local cerebral blood flow [6], which often has been used as a surrogate marker for the presence of CSD.

Electrical activity associated with CSD has been shown to occur only on the cortical surface and does not penetrate to deeper brain layers [7]. This led to the concept of this phenomenon being a local cortical event, although more recent studies have shown that marked changes can occur in sub-cortical nuclei, perhaps for a prolonged period of time.

Despite many years of research, understanding of the functional importance of CSD in human pathology has been very limited. Key points of concern have been the limited evidence of detection of spreading depression in the human brain that, when taken together with the general difficulties of generating CSD in higher species, resulted in a concept of spreading depression being only relevant to experimental animals.

Throughout the past few years, tremendous advances in technology, including imaging modalities, have allowed detection of spreading depression in the human brain. These observations are the key to highlighting the potential importance of this phenomenon in human disease. Recent studies also have detected the more indirect effects of CSD in humans, which indicate that it may play a role in human pathophysiology. The aim of this review is to highlight key advances in our understanding of the mechanisms associated with the phenomenon of CSD and to outline potential strategies for therapeutic intervention in migraine. It focuses on several key questions. First, does CSD occur in the human brain? Second, if CSD does occur in humans, is it specific to migraine aura? Third, can CSD contribute to activation of trigeminal vascular responses? From a perspective of therapeutic intervention, it also is

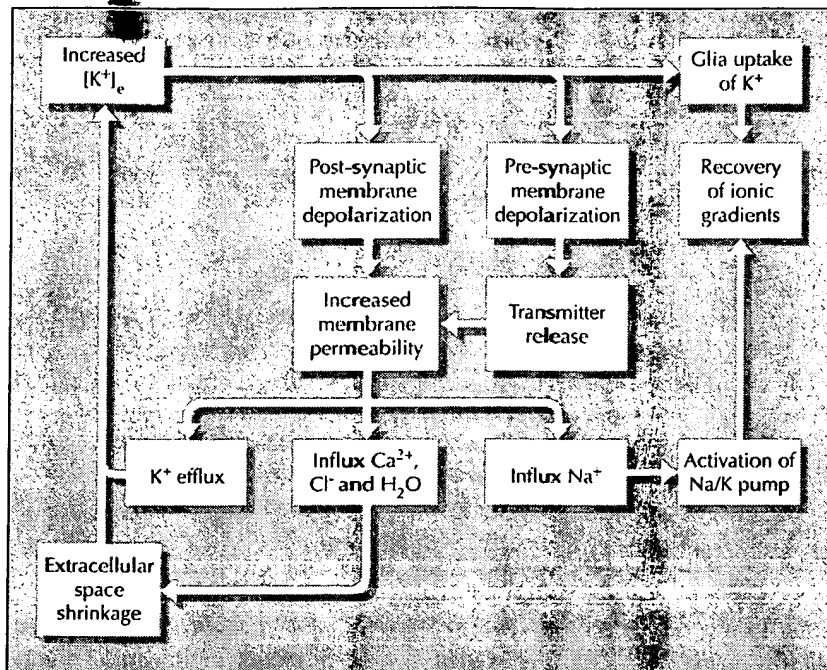


Figure 1. Cortical spreading depression. A schematic representation of the cellular events providing a balance of activation and repolarization during spreading depression.

important to answer the question of whether CSD occurs in a majority of patients with migraine. If CSD occurs in a number of patients, how can we target this potential disease mechanism?

Cortical Spreading Depression and the Human Brain?

Studies aimed at precipitation of CSD activity in the human brain have focused largely on patients undergoing surgical procedures, usually for intractable epilepsy [8] or traumatic injury [9]. This can be used as a model for other disease areas because traumatic injury may be considered to be an effective stimulus for induction of spreading depression and it gives rise to CSD-like activity in a range of animal species including primates [10]. Therefore, failure to show CSD-like events in the injured brain would provide considerable doubt regarding its importance in humans.

Unfortunately, extensive investigation in a significant number of individuals failed to demonstrate induction of CSD in humans [8], supporting the concept that CSD is only an experimental phenomenon. However, CSD activity has been detected in human hippocampal tissue *in vitro* [11], providing some support that CSD indeed can occur in the human brain. Some explanation perhaps could be provided from animal studies in a model of status epilepticus. In animals experiencing pilocarpine-induced seizures, there was a failure of induction and propagation of CSD events [12]. Although the precise explanation for these differences is not evident, a possible hypothesis could be that prolonged seizure activity had resulted in changing the induction threshold for CSD, which perhaps offers a partial explanation for the lack of CSD in man.

Other approaches have attempted to detect spontaneous CSD activity in the injured human brain. Initial studies aimed to specifically measure CSD in the human brain have used a multiparametric recording approach measuring $[K^+]_e$, dc potential, and electroencephalographic and intracranial pressure in patients with severe head injuries [9]. Using a single probe during routine neurosurgical monitoring, Mayevsky *et al.* [9] were able to detect CSD-like activity in only one of 14 patients. Although these findings are encouraging, a minimal number of CSD-like events were observed. A more definitive study using a linear array of recording electrodes was able to record from an approximate 4-cm strip of cortex in patients undergoing neurosurgical procedures following head injury or intracranial hemorrhage. This investigation was able to show episodes of decreased electrocorticography (ECoG) in five patients at a rate consistent with spreading depression [13•]. Transient depressions of ECoG with no evidence of spreading also were detected in eight patients [13•]. Taken together, this study provides evidence that CSD-like events occur at frequencies greater than previously detected and clearly support that CSD occurs in the injured human brain.

Does Cortical Spreading Depression Occur in Migraine Without Aura?

Cortical spreading depression is a dynamic process and to detect it with any methodology, one must be measuring at the right place and at the right time. Studies using diffusion-weighted magnetic resonance imaging (DWI MRI) in anesthetized cats have clearly shown that propagation of CSD events also may be spatially limited within individual gyri of the brain [14]. Use of DWI appears to be

a suitable surrogate for measuring electrical activity in the cortex because it will change in response to cell swelling induced by depolarization. In lissencephalic species, such as the rat brain, detection of CSD activity with this type of imaging modality is attained more easily because propagation of each event usually occurs across the whole cortex [15]. Therefore, from using data from animal experiments, one would predict that there is potential for CSD activity in humans to be highly localized within a specific cortical gyrus. Therefore, a high degree of temporal and spatial resolution will be required to detect CSD using DWI MRI in conditions without a specific reference point (eg, a head trauma). This may offer too many practical and ethical considerations for prospectively studying CSD in the human brain during spontaneous headache.

However, a serendipitous observation during a positron-emission tomography (PET) activation study in which a subject underwent a spontaneous migraine showed a classical spreading hypoperfusion of the cortex, initiated in the occipital lobe, and consistent with CSD [16]. Although blood flow studies do not measure neuronal activity per se, they could be considered a suitable surrogate measure of CSD. In the PET study by Woods *et al.* [16], the subject with a spontaneous headache did not report any aura symptoms; however, it is difficult to conclude that this indeed was migraine without aura. However, based on the limited data available, there would appear to be support for the concept that CSD can occur in migraine without aura, but this still requires definitive studies that also will need to address the spatial and temporal relationship of CSD and headache.

Migraine with Aura

In only 20% of migraine cases is the headache preceded by a slowly moving scintillation moving across the visual field followed by temporary blindness [17]. These visual disturbances are known as aura and appear to progress across the visual field at the same rate as spreading depression [18]. The clear similarities have led to strong association with CSD and aura [4,19]. Use of highly specialized equipment that allows detection of electrical disturbances on the cortical surface of the brain also provides supportive evidence for the concept of CSD occurring in aura. Magnetoencephalographic studies measuring small changes in magnetic flux density have been able to measure disturbances consistent with CSD in patients [20]. However, the complexity and high degree of specialization and cost of the equipment required for these studies make it unsuitable for widespread studies.

Assessment of changes in cerebral blood flow during migraine with aura have provided strong evidence of CSD occurring in humans. A functional MRI study provides highly compelling evidence for the initiation of a spreading neurovascular event occurring in the occipital cortex during aura [21•]. In this study, the bold response to an

on-off visual stimulus (flickering checkerboard) was assessed in an exercise-induced headache, with the patient being able to mark the onset of aura while in the magnet. Before the onset of the aura, increases in blood oxygenation level-dependent (BOLD) signal in the occipital cortex were detected in response to the "on" visual stimulus and the decreased signal was associated with the "off" signal. These responses were considered to be normal and similar to the interictal period. After the onset of aura, the BOLD activation pattern changed considerably and showed a decrease of the amplitude of the on-off signal oscillation and was associated with an increase in the mean signal. Perfusion-weighted images taken 1 to 2 hours after initiation of the attack also showed a decrease in regional cerebral blood flow. Topographic analysis was consistent with progression of the BOLD signals from central to peripheral visual fields with a velocity of 3 to 4 mm min⁻¹. These studies provide a highly detailed spatial and temporal resolution of the BOLD changes observed during migraine aura, which provides extension of previous studies focusing on measuring cerebral blood flow changes using a variety of techniques such as Xenon [22], single photon emission computed tomography [23], PET [16,24], and MRI [25-27].

Considering all of the evidence, it appears that a spreading neurovascular event very similar to CSD in animal models occurs during migraine aura. However, the functional importance of this process still needs to be elucidated. One also may agree that there is some direct evidence linking CSD to only a few patients with migraine, although there is considerable evidence for some cortical dysfunction in migraine. The hypothesis that CSD plays a role in these disturbances still remains to be tested.

Can Cortical Spreading Depression Modulate Trigemino-vascular Reactivity?

Initial studies by Leao [2] demonstrated that CSD induces marked vasodilatation of the pial circulation in animal models. Studies have shown a role of calcitonin gene-related peptide in mediating these responses [28-30], possibly in combination with the release of nitric oxide in certain species [29]. These observations supported the concept that CSD can produce local activation of the trigeminal system. Conclusive evidence was obtained from studies showing that a section of the trigeminal nerve resulted in a marked inhibition of CSD-induced vasodilatation in the rat [30], although these studies also indicated that parasympathetic nerve reflexes were involved in this response. Local blood flow responses to a variety of vasoactive stimuli also are influenced by the trigeminal nerve [31]. These data collectively indicate that the trigeminal vascular system can play an important role in modulation of cerebral arterial vasoreactivity. However, this may not indicate that local activation of the sensory nerve endings results in activation of the second- or third-order neurons

in this nociceptive system. Evidence for activation of the sensory pathways came from studies using c-fos as a marker of neuronal activation. CSD induced by application of microinjections of KCl produced activation of c-fos in regions of the trigeminal nucleus caudalis, effects that were blocked by ablation of the sensory nerve and by sumatriptan [32].

However, these studies were not supported by other workers [33]. In these studies, CSD-induced c-fos protein-like immunostaining was related to the number of evoked KCl injections, not to CSD events. The authors concluded that activation of sensory fibers was not related to CSD per se, but to hyperosmolar injection of KCl. This highlights that a number of confounding variables can make comparison between studies difficult. Other studies also were unable to detect activation of cervical spinal cord craniovascular sensory neurons following CSD [34].

More recently, the effects of CSD on trigeminovascular activity have been extensively studied using c-fos expression, protein extravasation, and changes in local blood flow [35••]. In these investigations, a delayed and long-lasting increase in meningeal blood flow was observed after induction of CSD by pin prick or electrical stimulation on the cortical surface. Peak changes in meningeal blood flow were detected 15 minutes after the induction of CSD, which returned to baseline within 45 minutes. These effects also were associated with reduction in cortical blood flow [35••]. Furthermore, the long-lasting changes in meningeal blood flow were blocked by the NMDA channel blocker, MK-801, section of the trigeminal afferent nerves and were markedly attenuated by a section of the postganglionic parasympathetic nerves, demonstrating a complex CSD-induced neurovascular reflex [35••].

This study also demonstrated that CSD produced protein extravasation in the dura mater and activation of second-order neurons in the trigeminal nucleus caudalis (Laminae I and II). As with previous studies, CSD-induced c-fos immunostaining was blocked by sumatriptan [35••]. These elegant studies confirm that CSD can produce complex neurovascular events and provides a mechanism by which extracerebral cephalic blood flow couples to intrinsic brain activity and may produce activation of pain pathways possibly resulting in headache.

Advances throughout the past 4 to 5 years have shown that spreading depression of ECoG activity can occur in the human brain [13•], confirmed that neurovascular events consistent with spreading depression occur in migraine aura [21•], and that CSD can modulate meningeal blood flow and trigeminal nociceptive pathways [35••]. This circumstantial evidence clearly would support a role of CSD in migraine headache. However, several underlying questions remain to be answered. Does CSD occur in most patients with migraine? Does CSD really result in headache in susceptible patients?

Migraine: A Central Sensitization?

There is building evidence from animal [36,37] and clinical studies [38,39•,40] for central or peripheral sensitization occurring in migraine. The presence of sensitization may even affect the efficacy of current therapies. In a study of 31 patients, 93% of migraine attacks without the presence of allodynia were rendered pain-free within 2 hours of triptan therapy. In contrast, only 15% of allodynic attacks were rendered pain-free during this time period [40]. In light of these new findings regarding the nature of the headache attack, it would appear that to fully understand the possible effects of CSD in migraine, studies should be directed toward whether CSD can induce sensitization of second- or third-order sensory neurons on the trigeminal pathway.

It is tempting to speculate on mechanisms by which CSD could modulate sensory pathways to produce sensitization. CSD can induce inflammatory cytokines [41] and central induction of cyclooxygenase-2 [42] mediators that may cause sensitization. Long-lasting changes in gene or protein expression also may occur after CSD. Changes in nitric oxide production for up to 14 days were noted after CSD [43] and a moderate elevation of cyclic guanosine monophosphate (cGMP) concentrations were detected 3 days after CSD in the mid-brain regions [44]. Although CSD is locally confined to the cortex, it can have widespread effects on central nervous system activity, possibly as a result of antidromic activation of neuronal pathways. CSD can modulate subcortical areas of the brain such as the locus coeruleus [45] and thalamus [46]. The effects of CSD on sensitization of nociceptive pathways remain to be studied in detail, but this may provide greater insight into the potential role of CSD in the generation of migraine headache.

How to Treat Spreading Depression

It could be argued that to completely understand the role of CSD in migraine, clinical studies using a specific modulator of CSD activity will provide the greatest insight. CSD may mediate aura and appears to have a stimulatory effect on trigeminal pathways; therefore, pharmacologic approaches to modulate CSD have focused on blocking its generation. Figure 1 illustrates a number of critical steps in the production of CSD in terms of depolarization and repolarization. These may allow targeting of pharmacologic approaches to inhibit the generation of CSD. For example, approaches that inhibit neuronal depolarization through the NMDA channel or block elevation of $[K^+]_e$ would inhibit neuronal depolarization and prevent the propagation of CSD. Examples would include use of the selective NMDA channel antagonist MK801 or glycine co-receptor antagonist L-701324 [47]. Intranasal ketamine can stop prolonged aura in some patients [48], although ketamine may not be suitable for routine use.

Other approaches used modulate neuronal cell activation and have had similar effects. For example, inhalational anesthetics such as halothane have a well-documented inhibitory effect on CSD induction [49,50]. In addition, some compounds with anticonvulsant effects have been shown to inhibit spreading depression. These include BTS72664 [51], furosemide [52], and SB-220453 [53,54], although not all anticonvulsant drugs possess these properties. Sodium valproate had no effect on CSD [50]. Other voltage-operated channel blockers also may have similar effects.

An extensive review of the effects of a variety of pharmacologic agents has been described by Marrannes *et al.* [55]. A review of a number of drugs used as antimigraine agents at that time showed that none attenuated CSD. Later studies were able to confirm that a range of antimigraine agents such as propranolol and sumatriptan had little effect [50] on propagation. Although ergotamine, which has 5-HT_{1B/1D} agonist properties, actually produced an increase in the duration of spreading depression [55].

Therefore, it would appear that a variety of approaches to limit cell activation may have inhibitory effects on CSD. Consistent with this hypothesis is the observation that enhancing neurotransmission or cell excitability may reduce the threshold for induction of CSD. Recent studies have shown that insertion of a calcium channel gene containing missense mutations associated with familial hemiplegic migraine appear to increase excitability in the mouse [56]. Insertion of the mutated CACNA1A gene into mice produced enhanced neurotransmission at the neuromuscular junction, a reduced threshold, and increased velocity of CSD [56].

Therefore, approaches that balance neuronal excitation and inhibition appear to be a useful approach to therapeutic intervention targeting CSD. However, many neuromodulator/transmitter systems may have dual activity and even have competing molecular mechanisms related to CSD. For example, it is thought that inhibition of nitric oxide production would be beneficial in the treatment of migraine because it may have vasoactive and direct effects on sensory neurons. It also may have some potentially beneficial effects on repolarization of central nervous systems neurons following CSD. Infusion of a nitric oxide synthase inhibitor lengthens the duration of CSD events [57]. These effects appear to be independent of cGMP as selective inhibition of the phosphodiesterase enzyme metabolizing cGMP, with zaprinast or sildenafil having no effect on nitric oxide synthase inhibitor-induced increases in the duration of CSD events [58]. This example highlights the complex interactions that may take place with a variety of biologically active substances involved in neuromodulation of the functional effects of CSD.

Depending on the precise mechanism under investigation, it is possible to envisage that drugs with these properties also may have anticonvulsant, antinociceptive, or limited sensitization of neuronal pathways. Although the

potential for multiple pharmacologic effects may make the conclusion of the specific role of CSD in migraine difficult, it makes it a more attractive strategy for drug discovery. Therefore, development of new medications or further understanding of the properties of existing drugs may allow a better understanding of potential avenues for the treatment of CSD.

Conclusions

Tremendous advances in understanding the potential role of CSD in the human brain function have occurred. CSD now can no longer be dismissed as an experimental artifact that does not occur in humans. However, we need to broaden our understanding of the precise role of CSD in normal brain function and its role in pathophysiology. There is no doubt that future technologic developments in imaging and measuring brain function will provide ways to elucidate the function of CSD. As our understanding of the mechanisms associated with the induction and propagation of CSD increases, new therapeutics can be designed. Hopefully, future research will bring answers to the many outstanding questions regarding CSD and provide better understanding of this enigmatic phenomenon.

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